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## BINUCLEATE CELLS IN CERTAIN HYMENOMYCETES.

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(WITH PLATE I)

INCLUDING the basidia and spores, Rosenvinge found that the cells of the Basidiomycetes contain from one to many nuclei. He was of the opinion that the uninucleated condition is typical and that only the uninucleated cells divide, the multinucleate condition arising secondarily by division of the originally single nucleus of each cell.

Two notes by R. Maire (**9**, **10**) in the *Comptes Rendus* of July 9 and December 24, 1900, report that the hyphal cells of the young carpophore in a large series of Hymenomycetes are regularly binucleated. This discovery throws a new light on the question as to the nature of the nuclear fusions in the basidium, and indeed on the whole question of the morphology of the carpophore. In view of the many times suspected relationship of the Basidiomycetes and Ascomycetes and the seemingly natural assumption of the equivalence of the nuclear fusions in the ascus and basidium, my own work on the ascus led me to desire further confirmation of the results given by Maire, and especially further light on the number and nature of the nuclei in the mycelium as well as the carpophore of the Hymenomycetes. In his first note Maire describes observations on a series of nine genera of Agaricineae, Polyporeae, etc. In all these genera he finds that the mycelial cells of the young carpophore

are binucleated. In the mature carpophore the majority of the cells in the stipe and pileus are multinucleate, but this condition arises from the amitotic fragmentation of the two nuclei originally present in each cell. The subhymenial cells from which the basidia spring and the paraphyses are always binucleate. This involves the remarkable fact that all the cells which are directly concerned in producing the basidiospores are throughout their development binucleated. In other words, all the cells of the *Keimbahn* are binucleated, the multinucleated condition only arising in cells of strictly limited development and found in the organs of nutrition, support, transportation, etc. Maire also finds that the pairs of nuclei divide simultaneously as "conjugate nuclei" (**12**), so that in the successive cell generations which arise in the development of the carpophore the two nuclei in each cell are always of widely separated nuclear ancestry, the whole condition duplicating exactly what has been described by Dangeard and Sappin-Trouffy (**3**) and Poirault and Raciborski (**12**) for the rusts. Further, in the young basidium two and only two nuclei are always present, just as in the teleutospore of the rust. These two nuclei fuse to form the primary nucleus of the basidium, which then divides twice to furnish the nuclei for the typically four basidiospores. The descriptions of Rosen (**13**) and Wager (**16**, **17**), according to which a series of nuclei fuse successively in pairs to form the primary nucleus of the basidium, are entirely incorrect according to Maire for the large series of forms examined by him.

In his second note Maire describes observations on a series of Gasteromycetes, in which he finds exactly the same conditions as described above for the Hymenomycetes.

Maire believes that the fusion of nuclei in the basidium is without doubt sexual in its nature, since the pronuclei which unite are of widely separated origin, although passing the whole course of their development in a single cell series. These facts bring the carpophore of the Basidiomycetes into closest parallelism with the whole life cycle of the Uredineae, both as to the nature of their vegetative cells and in the similarity of the

processes in the basidium with those in the teleutospore and promycelium. The only marked difference is in the fact that the promycelium of the rusts is a septate basidium with lateral spores instead of a one-celled basidium with apical spores. Should Dangeard's (**2**) claim that the vegetative cells of the so-called Protobasidiomycetes are binucleate be confirmed, a very complete parallelism would be demonstrated between the latter and the rusts of the Coleosporium type. Maire says little of the mycelia of the Basidiomycetes which he has studied. The mycelium of *Coprinus radiatus*, however, he says, has uninucleated cells; where the transition from the uninucleated to the binucleated condition occurs he does not say.

Maire describes at some length the division of the nuclei in the basidia. The pairs of nuclei as they divide in the mycelium have each four chromosomes. The fusion nucleus in the basidium shows four chromosomes, both in its first and second divisions. The accuracy of these points can be better judged when the author publishes his figures. He also describes a peculiar and interesting behavior of centrosomes at the time of the formation of the sterigmata. The centrosomes lie at the points on the basidial wall where the sterigmata are to bud out. From these centers fibers differentiated out of the cytoplasm extend to each of the four nuclei which lie at the base of the basidium. Under the influence of these fibers the nuclei gradually approach the vertex of the basidium.

In the light of Maire's observations it becomes probable that the observations of Strasburger (**15**), Rosenvinge (**14**), Istvánffi (**8**), and others, according to which the cells of the Basidiomycetes are regularly multinucleated, were based only on a study of the old vegetative cells of mature sporophores, whose multinucleated condition is secondary and developed in connection with their special enlargement as supporting cells for the variously placed hymenium. Wager says very little as to the nuclei of the vegetative cells and does not describe the condition of the subhymenial cells, though he describes the hyphal nuclei as wandering into the young basidium in pairs (**17**). His

descriptions relate mainly to the phenomena in the basidium, but he has figured (**17**, *pl. XVII, fig. 1*) part of a hyphal cell with a pair of nuclei, which from their position and size suggest that they may be the only nuclei in the entire cell.

An investigation, not yet published, of the structure and development of *Tremella mycetophila*, parasitic on *Collybia dryophila*, by a student in my laboratory, showed that the carpophore of the Collybia contained regularly binucleate cells.

The species of Hypochnus, owing to their simple structure and the loose branching habits of their basidium-bearing hyphae, are very favorable material for the investigation of the nuclear phenomena in the mycelium and young hymenium. Species growing on very rotten wood are specially adapted for good fixation and sectioning. A species agreeing fairly well with *Hypochnus subtilis* Schroet., except that the setae are brown, is abundant in this region. The material in all stages of development from vegetative mycelium to well-developed hymenium with ripe spores can be readily obtained in the fall months.

My material was fixed in Flemming's and in Merkel's solutions, and was sectioned and stained with the safranin-gentian-violet-orange method.

It became apparent at once, as was to be expected, that the cells of Hypochnus, which develops no carpophore of sterile tissue, were regularly binucleate throughout, with the exception of the old stalk cells of the cystids and perhaps the cystids themselves. The conditions in this respect duplicate as nearly as possible those in a rust of the type of *Coleosporium sonchi-arvensis*. As is well known, the genus Hypochnus includes a group of the very simplest known Hymenomycetes. The mycelium ramifies in the substratum of decaying wood or rich earth and produces a simple hymenial layer on its surface made up of basidia alone, or, in some species, with intermingled cystids. As the genus is described, there is nothing to represent a carpophoric structure.

The hymenium is itself quite loose. At first it consists merely of scattered, erect basidium-bearing hyphae, which are

readily isolated and show a basipetal habit of branching, very well represented in the figure (*137*, p. 301) of De Bary's *Handbook* (**4**). The successive development of such erect hyphae and their becoming interwoven and pressed together results in the formation of the hymenial layer. At first these erect fertile hyphae can be readily isolated and studied in the living condition, as was done by the older authors, the entire system of branches from a single main trunk being thus very easily and accurately worked out, as shown so well in the figure referred to above. In stained sections, too, these earlier tree-like fertile branches stand out with beautiful distinctness, and show basidia in all stages of development, and with their nuclei in all conditions. The order of succession in the development of the basidia can be traced with perfect accuracy. Later, as the hymenial layer becomes more dense, these individual twig systems can be no longer differentiated, though there is no question that the further development consists in nothing more than the pushing up of additional erect hyphal branches among those earlier developed. The species investigated forms a delicate frost-like growth on the surface of very well rotted logs, and should be collected in damp weather. The substratum was so soft as to offer no special resistance in cutting, and it was therefore possible to take off thin slices of the substratum several millimeters square and fix them with practically no disturbance of the fungus.

The mycelium was found ramifying through the decayed wood cells in all directions. The peculiar clamp connections, described by Hoffman (**7**) between adjacent cells of a hypha, were abundant. In fact, they seemed to be present at one or both ends of nearly every cell. Brefeld (**1**) has described these clamps in Coprinus as originating in a tube which pushes out from the end of one cell, bends over, and fuses with the end of the next adjacent cell. Brefeld finds that before the clamp tube fuses with the second cell it is separated from its parent cell by a septum. Later he finds also that a second septum is put in, replacing the walls broken down in the fusion. The mature clamp is thus cut off by a septum from each of the two

cells which it connects. I have not observed the development of these clamp connections in Hypochnus, but in the mature condition as they are universally found in my material they show but one septum, and that apparently on the cell from which the clamp-tube originated. The significance of these structures is not clear. Presumably they facilitate the transfer of excess food materials and moisture from one cell to another, but just how they are adapted to this function is not evident from their structure. I have not observed in Hypochnus the granules or plates on the septa between adjacent cells which are so common in many Basidiomycetes and Ascomycetes (**5**, **7**), and which probably indicate the location of some sort of pores for communication between the hyphal cells. In Coprinus, as we shall see later, such plates are present in great abundance, and have a very characteristic appearance.

The mycelial cells of Hypochnus vary considerably in length, though of fairly constant diameter in the mature mycelium. A cell of about average proportions is shown in *fig. 1*. In the specimens studied the hyphae formed no dense wefts or strands in the substratum. On the whole it seemed rather loose and sparse. The vessels of the decaying wood with their large empty cavities afforded a favorable opportunity for studying the hyphal cells, the hyphae cutting through these cavities in all directions, but never filling them densely. The mycelial cells are practically without exception binucleate. The two nuclei generally lie rather close together and near the center of the cell, but they may be at opposite ends, or otherwise placed in the cell. *Fig. 10* shows that, however the pair may be located in the cell, they are generally rather close together. These nuclei show, in well-stained preparations, a nucleole and chromatin sharply differentiated, and are sharply bounded by a nuclear membrane.

Fusions (**6**), such as have been described by many observers between cells that are near together or in contact, are quite common in Hypochnus. There is no clear evidence that in this case they have any other significance than as channels for the

interchange of food materials, etc. Such a fusion is shown in *fig. 10*, in the lower right-hand part of the subhymenial tissue.

The mycelium of Hypochnus in the case studied forms no felt of hyphae on the surface of the substratum, apparently, until the time has arrived for the formation of basidia. That is, through its purely vegetative existence it is entirely buried. When the hymenium is to be formed, erect aerial branches are pushed up, and branch into the tufts referred to above as figured by De Bary. The main axes of these tufts are never close packed, but arise from the substratum at relatively considerable distances from each other. Their abundant interlacing branches, however, form a dense hymenial layer higher up. We have thus a condition in this respect resembling a diminutive forest, or growth of shrubbery, whose branches and twigs are densely interlaced, while the trunks from which they arise are separated by relatively wide open intervals. This is shown, with a modification noted below, in the semi-diagrammatic *fig. 10*. The whole mass of the hymenium here shown is supported by the three hyphae arising from the substratum. The figure, to be sure, is of a section, so that part of the hymenial cells belong really to other twig systems arising above or below the level of the section. A corresponding portion of the branches of the two main hyphae figured lie also above or below the section, and hence do not appear, so that the proportion of peripheral branches to main axes as shown is not materially affected. The case is further complicated frequently by proliferation from the surface of an old and apparently exhausted hymenial layer. This is the condition shown in the figure in question, where the hymenium which shows the immature basidia has been superimposed on the surface of an older one. The upper surface of the latter is very clearly indicated, and it is at once seen that the same method has been employed in forming the second hymenial layer as in the first case. The old hymenium has become the substratum out of which arise scattering vertical hyphae, which by their copious branching then form the new hymenium. The old hymenium is seen to consist of a mass of hyphal cells very

poor in content which have replaced the basidia. The latter, as in all true Basidiomycetes, collapse soon after their spores are ripened.

Whether this habit of forming new hymenia by proliferation from those already exhausted is due to alternation of favorable and unfavorable conditions of moisture, etc., I am not sure. It is also uncertain whether a single hymenial layer may go on indefinitely producing new basidia to replace those which have ripened their spores as long as conditions are favorable for growth. Such continuous unlimited growth is quite possible by the basipetal method of branching shown in De Bary's figure. It would not result, however, in the formation of a second new hymenium, such as is shown in *fig. 10*, and it seems quite probable that the latter has been secondarily formed after a check to growth due to cold or drouth sufficient to stop the formation of basidia in the first hymenium. Whether a third hymenial layer might be developed in the same manner I have not determined. No such case has been found in the material so far studied.

It is interesting to note that, although the structures described for Hypochnus are extremely delicate and of microscopic size, the process of forming the new hymenial layers is essentially the same as that found in the larger stratified Polyporeae. Hypochnus, as we see, is really stratified, though not so described, and though lacking any markedly differentiated pileus. In the large woody fungi of the stratified Fomes type the new hymenial layers are formed as layers of pores and in successive seasons. But the method of their development by proliferation of new basidium-bearing hyphae from the old mycelial tissue of the previous season is essentially similar to that described above (so far as is known), except that the process is on a larger scale in Fomes, and the layers formed are of visible thickness.

If we look more closely now at the actual method of branching of the basidium-bearing hyphae of Hypochnus, we find, as indicated above, that the new branches are regularly produced basipetally. Each end cell becomes a basidium, and before this

apical basidium is more than half developed the cell beneath it pushes out a new basidium (*fig. 4*) which grows up beside the first. Later a second (*fig. 3*), and in some cases even a third, basidium may bud out on different sides and just beneath the basal wall of the first basidium. This gives the appearance of a stalk cell bearing a cluster of basidia at its apex. Since these basidia are pushed out successively, however, and their formation consists essentially in a process of cell division, the whole cluster represents really a basipetal series. The stalk cell becomes successively, at least as far as the nuclei are concerned, the sister-cell of each developing basidium as it is cut off. At the same time, from cells below the stalk cell of the first basidium lateral branches may be given off and grow to a length of from one to several cells, according to the level at which they arise from the main axis. Still further branchlets may arise from the cells of these lateral branches. In the end the apical cells of all these branches become basidia, and just beneath the septa which cut off these basidia one or two further basidia bud out. The basidium terminating the main axis of such a system is always the oldest and ripens first. The clusters produced on the lateral branches push up to the height of the main axis, but not higher, and ripen successively. The whole process is easily understood from a study of De Bary's figure and *figs. 2–4, 7, 9, 10.* With reference to their method of branching, we may compare such hyphal systems with their basidia to a flat topped cyme among the flowering plants.

The entire fruit body of Hypochnus is composed thus of a series of such cymosely branched hyphal systems, whose branches are intricately interlaced and packed together. Such a hymenium is strictly a compound structure made up of a series of similarly branching fertile hyphae. The only unity to be found in it lies in the fact of its possible origin from a single mycelium produced by the germination of a single basidiospore. As a matter of fact, it is highly improbable that such unity as this ever really exists in any given case. It is much more likely in the majority of cases that mycelia from different spores are

mingled in the substratum, and that any particular hymenial layer is made up of fertile branches from different mycelia. Their ability to combine into the even homogeneous films extending over many square centimeters would be wholly due to their similar environment and their mutual interaction, and not to any unity of origin, since each mycelium must be regarded as an independent plant.

It is plain that this conception of the Hypochnus hymenium as a compound having no strict individuality is of great importance for determining the morphological value of the more complex carpophores of the higher Basidiomycetes, and in comparing them with the fruit bodies of the Ascomycetes, which arise typically at least as units from the cell or cells of a sexual apparatus, so far as their fertile ascus-bearing hyphae are concerned.

The cells of the hyphae which occupy the place of the old hymenium in *fig. 10* are very poor in protoplasmic content. Among them are the empty and partly collapsed cell walls of the old basidia, which are scarcely recognizable, owing to the growth and crowding of the new mycelial tissue. How this new growth arises from the subbasidial tissue I have not been able to make out. The old basidia and their connections cannot be traced at all definitely at such a stage as is represented in *fig. 10*. There is no reason for anticipating anything especially noteworthy in the details of such a proliferation. The fact that it occurs, however, is a still further point of difference between the Ascomycetes and the Basidiomycetes. The former never show such secondary hymenia developed on the surface of old hymenia. The ascogenous hyphae show frequently a protracted, but none the less ultimately a definitely limited, growth, resulting in a characteristic fruit body, the ascocarp; while the merely vegetative hyphae which bear the basidia may prolong and repeat at intervals the development of new hymenial layers.

The stromata of Diaporthe, and other similar types which may show more or less secondary growth, are not ascocarps, but vegetative mycelia bearing ascocarps imbedded in their substance (**18**).

The cells of the old hymenium, like those of the mycelium buried in the substratum, contain regularly two nuclei. These are usually placed rather close together, as noted above. The apical cells which are to form basidia contain regularly two, and only two, nuclei. I have found no exceptions to this rule, though doubtless such occur as abnormalities, as pointed out by Maire. The young basidium is also regularly separated from its stalk cell at an early stage, so that in Hypochnus there is no chance for such a migration of pairs of nuclei as Wager thinks probable. The conditions are especially favorable in Hypochnus for determining this point. In the subhymenial tissue of the agarics the hyphae are so densely packed, in many cases forming a pseudo-parenchyma, that it is difficult to trace the path of a single hypha for any distance, or to determine just what the connections of the basidium at its base may be. In Hypochnus the loose branching makes it possible to determine just how and when each basidium has been formed and cut off, and it is certain for this case that Maire's general statement that two, and only two, nuclei are cut off in the young basidium is true, as opposed to those of Rosen (**13**) and Wager (**16**). The stalk cells also contain two nuclei. The conditions just described are well illustrated in *figs. 2*, *3*, and *7*.

Whether the two nuclei of the cell from which the basidium is cut off divide simultaneously, and whether simultaneous nuclear division ("conjugate nuclear division" of Poirault and Raciborski [**12**]) occurs generally in the formation of new cells in the mycelium, I have been unable to determine with certainty. That this is the rule in the case of the binucleated cells of the rusts there can be no doubt. The work of Dangeard and Sappin-Trouffy (**3**) and Poirault and Raciborski (**12**) establishes this fact and I have been able to confirm for myself their results on this point. As to the details of the so-called conjugate division, however, the figures so far must be considered as very unsatisfactory. The evidence presented by Sappin-Trouffy and by Poirault and Raciborski is not at all convincing on any points further than that the nuclei lie side by side and divide simultaneously.

In the same way I have also found great difficulty in obtaining satisfactory figures of the nuclear division in the cell from which the basidium is cut off, and prefer to reserve judgment on the whole process until more satisfactory methods of preparation have been worked out for this material. Maire in both his papers referred to describes the nuclear division as of the conjugate type, the same as in the rusts. Still the evidence is not clear, and in the formation of the secondary basidia, which bud out from the stalk cell of the one first cut off, figures are not uncommonly found which point to a different process. For example, in *fig. 4* it is seen that one nucleus is already in the young basidium, while one has remained in the stalk cell. It would seem as if the next step would be the division of these nuclei *in situ*, giving two sister-nuclei, for the young basidium and another pair of sister-nuclei for the stalk cell.

The stalk cells contain quite regularly two nuclei after the basidia have been cut off (*figs. 2* and *7*). I have also found a number of cases in which the two nuclei of the mother-cell of the first basidium of a cluster were dividing simultaneously. The figures, however, were dense and unsatisfactory, resembling the figures of the conjugate division in the rusts referred to above; and, while affording evidence of simultaneous division, are not, in my opinion, to be regarded as at all typical. Maire states that all the Hymenomycetes studied by him show four chromosomes in these mycelial nuclei, but in the absence of figures it is difficult to judge of the evidence on which this conclusion is based.

The pair of nuclei in a young basidium grow rapidly with the growth of the basidium. This very notable increase in their size seems to have been regarded by Wager as suggesting the likelihood of repeated nuclear fusions, but the phenomenon is not different from that in Synchitrium, where with the growth of the fungus cell the nucleus grows until from being one of the minutest of nuclei it becomes much larger than the nuclei of the host-plant cells which surround the fungus. The significance of such changes in nuclear volume is not clear, but they

are regularly correlated with increased size in the cell to which the nucleus belongs.

Fusion takes place generally when the basidium has reached from one-half to two-thirds of its mature size, though wide variations from this rule may be observed. The process can be observed readily in all stages. The nuclei become flattened upon each other. The nuclear membranes disappear over the area of contact and the nuclear cavities become continuous. The fusion nucleus shows a constriction for some time around the area of union. This furrow gradually disappears and the nucleus is evenly oblong oval in outline. Later it becomes more spherical, regaining the shape which it had before fusion took place.

Just how the chromatin masses are combined cannot be made out. They become apparently continuous in the region of contact as soon as the membranes have disappeared. Later no line of demarcation between the two can be made out. Still I am inclined to believe that the appearances of homogeneity are not to be taken as conclusive evidence on this point. There seems little doubt that the nucleoles fuse. They are conspicuous, sharply defined objects clearly differentiated as clear red spheres in the blue chromatin throughout the whole process of fusion. Each of the nuclei before fusion appears with astonishing regularity as possessed of one nucleolus (*figs. 2–4*), and just as regularly the spherical fusion nucleus shows a single and frequently much larger nucleolus. During the earlier stages of fusion the two nucleoli are still present (*figs. 5, 6*). They are frequently found later close together and pressed upon each other, though such figures as these are not to be considered as having great weight in view of the irregularity in shape which nucleoli frequently show. The fusion nucleus increases still further with the further growth of the basidium. It lies generally in the mature basidium at about two-thirds the distance from the base to the apex.

The stages in the division of the nucleus have been very fully described by Wager. They are abundant and easily found

in Hypochnus. In general, the karyokinetic figures agree with those found in the division of the ascus nuclei. A more detailed account of the stages I shall give later in another connection. The fusion nucleus regularly migrates to the apex of the basidium before it divides. The spindle is always transverse to the long axis of the basidium. In the equatorial plate stage and a little later the chromosomes are well individualized on the spindle, and the number seems to be at least from eight to twelve before the anaphases begin, as shown in *fig. 8*. Maire gives four as the number of chromosomes appearing in the division of the nucleus of the basidium in the long series of forms studied by him. It is certain that there are many more than four chromosomes in the nucleus of the basidium of Hypochnus. The second division immediately follows the first, and the spindles here are also transverse to the long axis of the ascus.

The four daughter nuclei then retire downward to about the middle of the basidium, while the basidium stretches up still farther in length, and the sterigmata bud out around its apex (*fig. 9*).

Maire has described strands of fibers (kinoplasm) connecting each nucleus with the point of origin of a sterigma. He interprets this as signifying the presence of centrosomes on the walls of the basidia at the point at which the sterigmata are to form, and considers that the fibers are concerned in drawing the nuclei up into the spores which bud out on the tops of the former. I have observed in some cases in Hypochnus such fibers extending from the nuclei up into the developing sterigmata, but their origin I have not been able to determine. The fully formed spores contain a single nucleus and are oblong, and, as a rule, slightly flattened on one side.

I have also investigated the nuclear phenomena in a species of the Agarics, *Coprinus ephemerus*. Sections through the young fruit body reveal at once the conditions described by Maire. The stipe and pileus consist of large multinucleate cells with extremely numerous nuclei. A cross-section of a small group of cells from the stipe is shown in *fig. 12*. The cells in cross-section

are seen to be almost circular, and of extremely variable size. The entire cross-section of the stipe shows it to be made up of large cells, with very much smaller cells intermingled with them. The center of the stipe in these species is hollow, and the hollow space is bounded by a layer of these smaller cells. A longitudinal section of the stipe shows that the cells shown in *fig. 12* are immensely elongated and quite regularly parallel, and in turn are combined end to end to form parallel hyphae.

These sections show also that the cells contain very numerous nuclei, the number varying with their size. In very young carpophores the cells are quite rich in protoplasmic content, the protoplasm being largely aggregated in a central column running the length of the cell, in which most of the nuclei lie. As the cells elongate with the growth of the carpophore, they become proportionally poorer in content, and the nuclei are distributed more evenly.

The tissue of the pileus is also seen to be made up of large multinucleate cells. *Fig. 13* represents a group of cells from the peripheral region of the pileus, and shows four cells prolonged into blunt, spine-like processes, which project outward from the upper surface of the cap. The whole surface of the cap is covered with similar spines in this species. Between these spine-cells are the columnar outer cells of the pileus, forming a sort of epidermis, and beneath these again are mingled large and small cells. All of these different types of cells are multinucleate, but are not as large and do not contain as numerous nuclei as the stipe cells. They are also relatively poor in protoplasm, and contain a single large central vacuole, or numerous smaller ones.

It is doubtless the study of these cells of the vegetative part of the carpophore that has led to the conclusion of Strasburger, Wager, and others, that the vegetative bodies of the Basidiomycetes contain typically many nuclei. If we study the cells which are forming the gills of a carpophore of Coprinus, however, an entirely different condition of things is found. The gills originate as plates on the inner surface of the pileus as soon as it is differentiated around the upper end of the stipe. The separation

of the pileus from the stipe is, indeed, brought about, in part at least, by the development of these plates. The first formed gills are attached along their entire margin, for a time at least, to the surface of the stipe, and the cavities lined by the hymenial surfaces originate as thin, vertical, intercellular spaces, placed radially about the stipe, as is very well shown by transverse sections through the upper end of the young carpophore. These earliest formed gills later split off at their inner edges from the stipe as the pileus expands and gradually assumes the umbrella shape. The intercellular spaces, which are the interlamellar spaces, become wider with the growth of the pileus, and later formed gills arise in these spaces as further outgrowths from the under surface of the pileus. The study of the nuclei in these later formed gills is especially easy. The mature gill consists of two hymenial layers, with a vegetative supporting layer between them, the trama. In the young gill these parts are not sharply distinguished. It is formed simply as a ridge made up of hyphal branches, which grow radially inward and slightly downward. I am inclined to think that these hyphae branch just as do the hyphae in the hymenium of Hypochnus, in something like a cymose fashion, the apical cells being continually pushed to the side by branches produced from the cell beneath. The growth of the hyphae progresses radially inward and downward until the width of the mature gill is reached. The branches which are pushed out right and left terminate their growth almost immediately by the formation of basidia. These basidia intermingled with sterile cells, which are apparently their morphological equivalents, together make up the hymenial layers. In the young gill the hyphae are packed densely together throughout. As they grow older the central cells become separated by intercellular spaces in many cases, and thus form the looser tissue of the trama.

Since the axis of growth of the gill is radially inward and also downward, sections in an exactly transverse plane through the young carpophore do not show the method of growth as well as those which are slightly oblique. Such sections, inclined a few degrees to the long axis of the carpophore, lie in the axis

of growth of the hyphae of the gills and give the largest percentage of cells cut longitudinally in the young gill. This is of course true for the young gills on one side of the carpophore only, those on the opposite side, as will be readily understood, showing the hyphal cells cut more nearly transversely than in exactly transverse sections of the entire carpophore. *Fig. 11* shows the section of a very young gill taken from such an oblique section of a carpophore. The cells are nearly all cut longitudinally, and the method of their growth and branching is fairly well indicated. Still even here certain hyphae are seen to have turned up or down and out of the plane of the section, so that the axial hyphae of the base of the gill and the end cells are not connected to form a continuous hypha in the section.

The conspicuous fact here is that all the cells of the gill are binucleated. Following backward through the base of the gill, we should pass suddenly into tissue of the pileus exactly similar to that shown in the inner portion of *fig. 13*. It is not easy to trace a single hypha from the one tissue to the other; still there seems no doubt that the binucleated cells are the end ramifications of hyphae which farther back in the pileus and stipe are composed of multinucleated cells. In other words, as Maire's work shows, the ends of the hyphae which are to reproduce the organism by means of basidia and basidiospores have maintained the binucleated condition, while the mass of the hyphal cells, having only the functions of support and transportation of water, etc., to the reproductive cells to provide for, have become enlarged and multinucleated as a result of nuclear divisions unaccompanied by cell division. The reproductive series of cells, the *Keimbahn* of various authors, is thus sharply distinguished from the purely vegetative and somatic series by the number of nuclei which they contain respectively. In very early stages in the development of the carpophore the differentiation of the two types of cells is present and continues through its whole development. Whether the multinucleated cells increase in number by cell division, as do the binucleated, is not easy to

determine. It seems highly probable, however, that, like the internodal cells of Chara and Tradescantia, they may have lost the capacity to divide, the increase in number being entirely provided for by the division of the binucleate cells. The development of the carpophore, as pointed out by De Bary (**4**, p. 49) and others, is no doubt to be interpreted as consisting in the growth of hyphae, and it seems highly probable that the hymenium represents the end cells of the hyphal branches which are to be traced back through the pileus into the stipe, and so connected with the mycelial branch or branches from which the carpophore sprung. The growth would then be largely apical for these hyphae, the older cells lying in the pileus and stipe, and the youngest, latest formed, in the hymenium. That all the hyphae of the stipe have end cells in the hymenium is, of course, not necessarily assumed, though it is not improbable, in view of the wide expanse of the latter, that this is the case.

What the condition of the mycelial cells is in the species of Coprinus studied I have not been able to determine. Maire says that the mycelium of *Coprinus radiatus* is made up of uninucleated cells. The base of the stipe of *C. ephemerus* is surrounded by a mass of hyphae with multinucleated cells; whether these, however, represent the condition of the primitive mycelium from which the carpophore sprung I am by no means sure. It may well be that in the work of collecting and transporting material for the young carpophore the mycelial cells may have become enlarged and secondarily modified as to the number of their nuclei. There seems little doubt, however, that the condition in Hypochnus represents a very primitive condition for the Basidiomycetes, and that, as Maire maintains, all multinucleated cells in their vegetative bodies have been secondarily produced. How the condition of things in *C. radiatus* with its uninucleated mycelial cells is to be connected with that in Hypochnus is not plain. It is quite possible, of course, that the uninucleated condition represented in the basidiospore may be perpetuated for longer or shorter periods of development in different cases.

The subhymenial cells of *C. ephemerus* show very conspicuous disc- or saucer-shaped bodies arranged in pairs on opposite sides of their walls. These bodies stain deeply and are very numerous, lying on all sides of the hyphal cells wherever the walls of adjacent cells are in contact. *Fig. 14* shows two pairs of such bodies and their relative position with reference to the cell walls. Strasburger (**15**, p. 335) has described similar structures. They doubtless mark points where the walls are perforated in some fashion, thus establishing connection between adjacent cells. I have described such bodies for Pyronema (**5**), and also spherical granules which may have a similar significance.

Brefeld (**1**) has described the carpophore of *C. stercorarius* as arising from a single hypha of the vegetative mycelium. This hypha produces a richly developed system of branches which arrange themselves ultimately into the parts of the young carpophore. Vegetative growth of the mycelium and formation of the carpophore are thus seen in this case to be rather sharply separated, and it might well be that at this point the transition from uninucleated to binucleated cells is effected. However, no such differentiation of mycelial growth and carpophore formation exists in Hypochnus, whose mycelium is binucleated throughout in its mature condition. Maire says nothing as to the stage when the binucleated cells first appear in Coprinus. If his statement is true, that the mycelium of the latter plant is made up of uninucleated cells, it differs, as noted, from Hypochnus in this respect. Whether Hypochnus or Coprinus would represent the more primitive condition in this particular is not easily to be determined with certainty. Most mycologists, however, have agreed so far in regarding the Tomentelleae as primitive Basidiomycetes, and the Agarics as among the most specialized forms; and, in the absence of positive evidence to the contrary, this seems the most natural assumption. Still, it is quite possible that the Hypochnus type may be degenerate in certain particulars and may have lost a mycelium of uninucleated cells, such as Maire reports for *Coprinus radiatus*. Just where in this case the

binucleated condition would arise is not clear. There is little doubt, however, that the development of multinucleated cells in the group is correlated with the origin of large and complex carpophores, and that in lacking such cells Hypochnus represents a more primitive type.

Basing his conclusion on the resemblance of certain conidiophores of the Ascomycetes to the so-called protobasidia, Massee (11) seeks to connect the Basidiomycetes with the Ascomycetes. He believes that it is in asexual stages of the latter that the ancestors of the Basidiomycetes are to be found, accepting Brefeld's conclusion that the basidium is a conidiophore which has become determinate in the number of spores which it produces. Massee points out that the conidiophores of these asexual forms of the Ascomycetes conform very closely to the type of the Auricularia basidium. From the fact that about thirty species of the genus Stilbum and several species of the genus Tubercularia are known to be the asexual reproductive forms of species of the ascus-bearing genera Nectria and Sphaerostilbe, while other species of the former genera have never been found connected with ascus-bearing forms, Massee argues that these latter asexual forms are probably becoming independent and have lost the ascus-bearing stage permanently. The conidiophores of the Stilbum form of *Sphaerostilbe microspora* Cooke and Massee are already typical protobasidia. The same is true of *Tubercularia volutella*, whose so-called conidiophores are likewise protobasidia. The examination of a large number of forms of Stilbum and Tubercularia shows that a series of gradations from forms with typical protobasidia, *i. e.*, two or three short swollen fertile cells, to forms with unmodified conidiophores, *i. e.*, two or three long narrow fertile cells forming the end of the conidiophore, can be made out. As a rule, the conidiophores are found on forms which are still associated with an ascus-bearing stage. *Sphaerostilbe microspora*, however, is an exception to this rule. Isaria, many of whose forms are connected with Cordyceps, while others are, the author believes, independent species, shows also typical protobasidia in *I. pulcherrima* Berk and Broome; while

other species show the transition from protobasidium to conidiophore. Massee believes that Möller's protobasidiomycete *Pilacrella delectans* stands close to the Isaria forms. Massee concludes that the "protobasidiomycetes as a group are derived from the conidial phase in the life-cycle of ascigerous fungi; the evolution is effected by the disappearance of the ascigerous form of reproduction, whereby the conidial phase assumes the standard of a species; this change being contemporaneous with the gradual conversion of the so-called conidiophore to the typical basidium or spore-bearing organ." He does not accept Juel's proposition to transfer such forms as *Stilbum vulgare* Tode into the Protobasidiomycetes, holding that the transition stages are so characteristic that no hard and fast line can be drawn between the forms with protobasidia and those with conidiophores.

Massee follows Möller in denying that the true basidium with apical spores has been derived from the septate basidium. He believes that these non-septate basidia have been developed from conidia of the type seen in Matruchotia, Botrytis, Polyactis, and others, through the non-septate basidium with lateral spores of the type seen in Tulostoma. Brefeld's Auto- and Protobasidiomycetes are both derived from the Ascomycetes, but as separate and distinct offshoots. Whether each of the two groups is to be further regarded as polyphyletic and consisting of separate series derived respectively from the forms Stilbum, Isaria, etc., the author does not state.

In the light of the facts described by Maire and myself, as recorded above, all such conclusions as these of Massee are seen to be extremely uncertain. The typical binucleated condition of mycelial cells is as yet unknown in the Ascomycetes, and until a transition from the coenocytic to the binucleated condition has been discovered in the forms discussed by Massee, his argument can be regarded as of little value. The widespread occurrence of regularly binucleate cells in the Basidiomycetes, with the additional evidence that these cells reproduce by conjugate division and constitute the reproductive series (*Keimbahn*) in

each individual through at least a considerable part of its life-history, leading up to the formation of basidia, while no such binucleated cells are found in the Ascomycetes either in vegetative or ascogenous hyphae, shows that the two groups are widely separated phylogenetically. In the face of such differences, resemblance of outer form and method of spore-formation between conidiophores and basidia must be regarded as superficial and of uncertain value, and as wholly inadequate evidence for the conclusion Massee wishes to draw. On the other hand, it is quite clear that the binucleated condition in the hyphae of both groups stlll further strengthens the evidence for the relationship between the rusts and Basidiomycetes. The nuclear fusions occurring in the teleutospore and basidium are seen also to be directly comparable processes, and there is a strong presumption that in the Basidiomycetes, as in the Uredineae, this fusion is not between sister nuclei, but nuclei which by the process of conjugate division have remained distinct through long periods of vegetative growth. Maire affirms unequivocally the existence of conjugate division in all the forms he has studied.

It must not be assumed without further evidence that the rusts are primitive Basidiomycetes. Their parasitic habit is against this view, and the evidence from the resemblance of a sorus of Coleosporium to a true hymenium cannot be considered as very conclusive. Still, the binucleate condition of the hyphal cells suggests very strongly that rusts and Basidiomycetes must have arisen from some ancestral type characterized, at least in some stage of its development, by the possession of binucleate instead of uninucleated or multinucleated vegetative cells. How this binucleated condition arose originally is not at all clear. The view of Raciborski that the binucleated cells of the rust represent a prolonged vegetative stage interposed between two phases of a sexual act, namely the cell fusion and the nuclear fusion, is suggestive as a hypothesis, but it is nothing more.

The question as to the nature of the spermogonia and their relation to the aecidia of the rusts remains still unsettled, and

De Bary's view as to the relationship of the rusts and Ascomycetes must be given up.

Further, it is plain, in view of the above facts, that the processes of nuclear fusion in asci and basidia are, so far as the present evidence goes, entirely unrelated morphologically, and that ascus and basidium are not to be brought into any common category as oogonia, as is attempted by Dangeard. Neither the cells of the ascogenous hyphae nor the vegetative mycelial cells of the Ascomycetes are binucleated in any case so far investigated, and there is no suggestion of any close morphological resemblance between ascus and basidium. The significance of the fusion is not entirely clear in either case, but no light is thrown on this point by endeavoring to bring together such heterogeneous structures.

It is highly probable that the nuclei of the basidium, as of the teleutospore, are of widely separated ancestry. It is clear in the case of Pyronema, at least, that the nuclei which fuse in the ascus are not sister nuclei. Further than this the resemblance in this respect does not extend. The origin of the ascocarp in a sexual process is analogous to nothing which has so far been discovered in the Basidiomycetes. It may well be that both the origin and the physiological significance of the fusions in ascus and basidium are entirely distinct.

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## EXPLANATION OF PLATE I.

All figures were drawn with the aid of the camera lucida, *figs. 1–4*, *7*, and *9* with the Zeiss apoch. objective ap. 140, oc. 8, and *5*, *6*, *8*, and *14* with oc. 18.

*Hypochnus subtilis.*

FIG. 1. Hyphal cell showing two nuclei and the characteristic clamp connections.

FIG. 2. Young basidium and stalk cell, each with two nuclei.

FIG. 3. Basidium with two younger basidia budding out beneath it.

FIG. 4. Young basidium with two nuclei; stalk cell with young basidium budding out and containing a single nucleus.

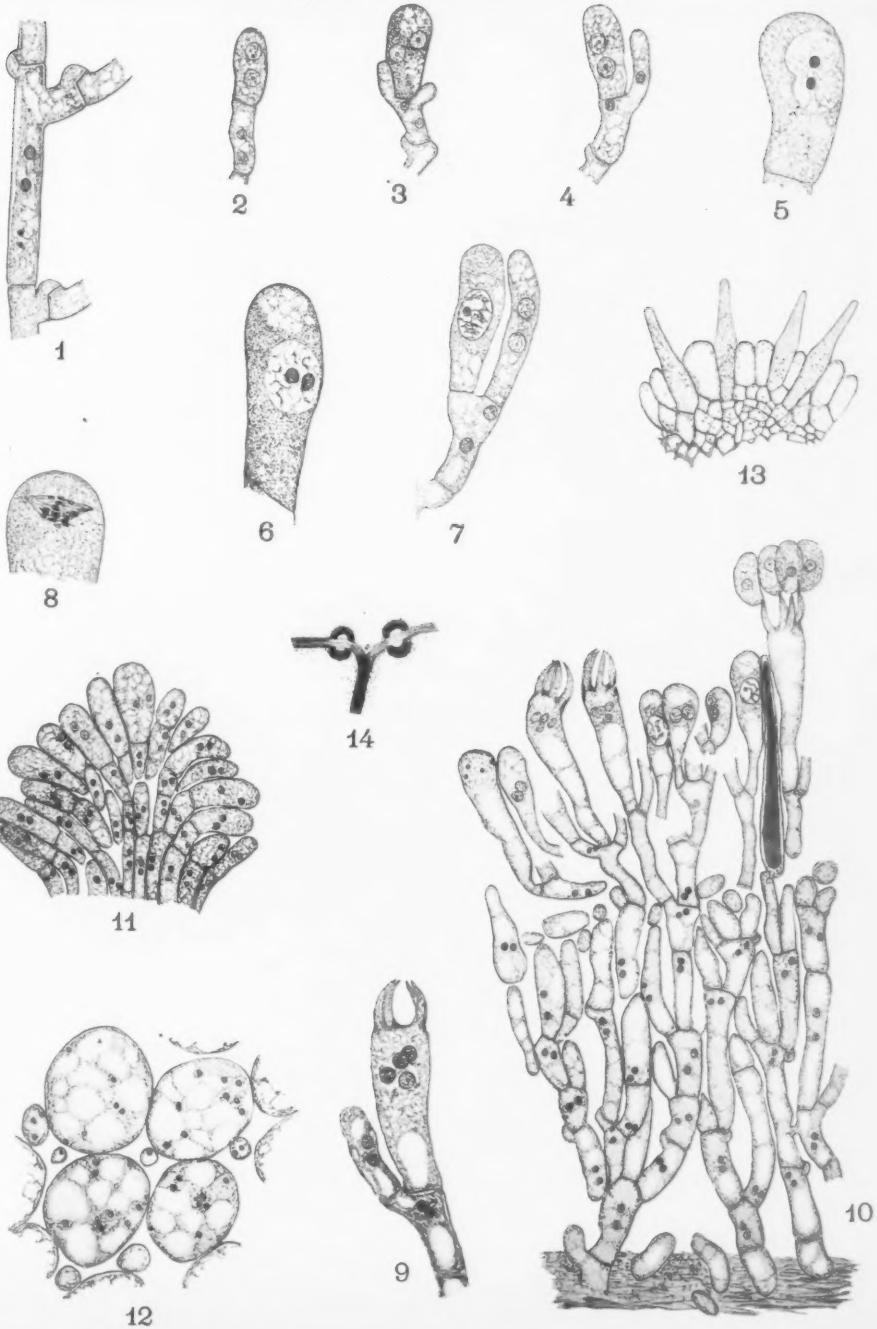
FIG. 5. Young basidium with its two nuclei fusing.

FIG. 6. Later stage in the fusion of the nuclei of the basidium.

FIG. 7. A pair of basidia in one of which nuclear fusion has taken place, while in the other it has not yet begun.

FIG. 8. First division of the nucleus of the basidium.

FIG. 9. Two basidia, one with four spore nuclei and sterigmata, and one with two nuclei before fusion.




HARPER on BINUCLEATE CELLS.

FIG. 10. Semi-diagrammatic section of the aerial portion: the upper layer is a young hymenium with basidia in various stages of development; to the right is a basidium with nearly ripe spores, each with a single nucleus; next to this basidium on the left is a young, only partly grown cystid, with very densely stained content; below the hymenium is a layer of vegetative tissue which has replaced a former hymenium; this is borne on three hyphal branches springing from the substratum below. × 800.

*Coprinus ephemerus.*

FIG. 11. Section of young gill made up of binucleate cells. × 800.

FIG. 12. Transverse section of a group of cells from near the upper end of the stipe of a very young fruit body. × 1000.

FIG. 13. Section of a bit of the peripheral tissue of the pileus, showing the columnar outer layer and four of the elongated papilla-cells.

FIG. 14. Parts of the walls of three sub-hymenial cells showing the pairs of bodies marking the position of intercellular pores.

# ON THE TOXIC PROPERTIES OF SOME COPPER COMPOUNDS WITH SPECIAL REFERENCE TO BORDEAUX MIXTURE.

JUDSON F. CLARK.

(WITH SEVEN FIGURES)

WITHIN recent years copper has come to be the most valued agent in combating the ravages of plant diseases. Its importance from an economic standpoint has attracted to its study an unusually large number of investigators, and as a result there is a very extensive literature on the toxicology of copper compounds. The object of the present study was to determine more exactly the concentration of the various copper compounds required to inhibit germination or to kill the spores of a fairly large and quite representative selection of fungi grown under favorable conditions; to determine the effect of various media on the toxicology of copper; and to throw light on the toxicology of the Bordeaux mixture. I shall not attempt to review the literature of the subject, except to refer very briefly from time to time to a few studies which are of special interest in connection with points in the present study. Those especially interested will find work before 1896 quite carefully reviewed by Fairchild,[1] Swingle,[2] and Evans.[3] References and reviews of work since that time may be found in the various reviews. Hollrung's Jahresbericht[4] is especially useful in this connection.

[1] FAIRCHILD, D. G.: Bordeaux mixture as a fungicide. Bull. no. 6. Div. Veg. Path. U. S. Dept. Agric. 1894.

[2] SWINGLE, WALTER T.: Bordeaux mixture, its chemistry, physical properties, and toxic effects on fungi and algae. Bull. no. 9. Div. Veg. Phys. and Path. U. S. Dept. Agric. 1896.

[3] EVANS, WALTER H.: Copper sulphate and germination. Bull. no. 10. Div. Veg. Phys. and Path. U. S. Dept. Agric. 1896.

[4] HOLLRUNG, M.: Jahresbericht über die Neuerungen und Leistungen auf dem Gebiete des Pflanzenschutzes. Berlin. 1898 and 1899.

Copper occupies an intermediate position in regard to toxic value for the mold fungi.[5] It is surpassed more than a hundredfold by mercuric chlorid, silver nitrate, and potassium chromate and bichromate. It is a little less toxic than nickel and a little more so than cobalt; distinctly more toxic than iron and the mineral acids; twelve times as toxic as zinc; and about eighty times as toxic as ethyl alcohol. Its great superiority as a fungicide lies in its cheapness, the tenacity with which its hydroxid, when properly prepared, adheres to the foliage of plants, and, as I shall show later, the solubility of its hydroxid in the presence of or by means of substances found alike in the cell sap of the host plant and of the parasite, but more especially the latter.

For a detailed description of the method of using the Van Tieghem cell for work of this kind the reader is referred to my article in a former issue of this journal.[6] I may add that the method has proved eminently satisfactory for various physiological and toxicological studies with pure cultures of fungi, yeasts, and bacteria.

Precautions for sterilizing were observed throughout the study, except with a few cultures of hymenomycetes. The spores used, of such fungi as can be normally matured in artificial culture, were taken from pure cultures frequently renewed to insure high vitality. Rhizoctonia and currant cane blight cultures were inoculated with mycelium taken from fresh pure cultures. With Hypholoma and Coprinus,[7] spores taken from plants growing under natural conditions were used. Naturally, every precaution was taken to avoid contamination, and on the whole very little difficulty was experienced on this account.

It is very important in any quantitative study of this kind to

[5] CLARK, J. F.: On the toxic effect of deleterious agents on the germination and development of certain filamentous fungi. Bot. Gaz. **28**: 289–327, 378–404. 1900.

[6] CLARK, J. F.: Bot. Gaz. **28**: 293. 1900.

[7] I beg to acknowledge my indebtedness to Dr. Margaret C. Ferguson, of Cornell University, who supplied me with the spores of Hypholoma and Coprinus, and to whose studies in the germination of the hymenomycetes I am indebted for the knowledge that they would be satisfactory for germination purposes in the media used.

have as nearly uniform a quantity of spores in the various cultures compared as possible. It is equally important to avoid bunches of spores in making the inoculation. Oversight in regard to either of these points is bound to result in more or less erratic and unreliable results. This is due to the fact that a toxic agent like copper, which effects the death of the spores by uniting with and precipitating the protoplasm, is itself also precipitated in the process and can no longer exert a deleterious influence. This precipitation process is naturally a strictly quantitative matter, and the amount of copper sufficient to sterilize a culture containing fifty spores may prove entirely inadequate were the number of spores quadrupled or even doubled. When bunches of spores are present the effect is still more mischievous. These bunches are relatively slowly penetrated by the copper-containing solution. Should the bunch contain air, as is usually the case, the process of penetration of the copper to the innermost spores is still further retarded. In the meantime such spores as are floating singly are quickly killed, precipitating their share of the copper, and it may be that long before the solution has fully penetrated the bunch it may have lost the greater part of its soluble copper and may be no longer able to kill the remaining spores. These may then germinate and grow almost as well as if no poison had been present. The difficulty was entirely overcome by soaking the spores for a longer or a shorter time in sterile distilled water with an occasional shaking. In some cases this soaking may be continued with advantage for days, in which case it is well to keep them at a low temperature to prevent germination. In all cases it was advisable to inoculate from a mixture of spores in water, a uniform inoculation being approximated in that way without great difficulty. It is equally important to have the culture drop of as uniform a size as possible. Absolute precision in these matters, however, is impossible, and the reader is reminded that slight variations in all cultures are to be expected. Indeed, as has been often suggested, spores taken from the same sporangium and placed in the same culture under absolutely similar conditions vary very

markedly in their capacity to resist poisons and other unfavorable conditions.

I would emphasize the importance of considering the *point of inhibition of germination* rather than the *death point* in consulting these results. The former can be determined with ease and absolute certainty by direct observation under the microscope, the latter only by transferring the spores to a fresh culture free as possible from the deleterious agent, and determining their success or failure to germinate under these conditions. The unavoidable source of error here is the transference of more or less of the copper compound with the spores. This quantity is doubtless often sufficient to prevent the germination of spores which although greatly reduced in vitality may be still alive. The general result of such transfers made at varying intervals from twenty-four hours to ten days indicated that if the germination of the spores be *inhibited* by a concentration of a copper salt, it was merely a question of time when they would be killed. Thus if a certain concentration inhibited the germination of a certain spore, possibly a solution of four times this concentration would fail to sterilize the culture in twenty-four hours, yet if left for some days the points of inhibition and death would finally coincide. The length of time necessary to bring this about would naturally vary with the varying specific characteristics of the spores of the different species.

The medium used for the cultures, the results of which are given in *fig. 1*, was a decoction of sugar beet. The root, sliced thin, in the proportion of 450$^{gr}$ per liter, was heated in an autoclave for an hour at 120° C., under a pressure of one atmosphere. The decoction was then filtered and poured into flasks and thoroughly sterilized. This medium was selected for the comparative tests as giving the best results with the greatest number of the fungi used. The other vegetable decoctions were prepared in essentially the same manner.

Stock solutions of the various copper compounds were prepared by a responsible chemist. A *normal* solution of a copper compound means in this paper the number of grams of the salt

corresponding to the full molecular weight (*e.g.*, $CuSO_4 \cdot 5H_2O = 249$) dissolved in sufficient water to make one liter of solution; thus 0.01 *n* $CuSO_4$ equals a 0.249 per cent. concentration of the *crystallized* salt.

In the figures used to illustrate this paper the solid black bar indicates that the concentrations used were sufficiently strong to kill the spores within twenty-four hours. The square blocks alternating with the open spaces indicates that while the germination of the spores was inhibited, they still retained vitality enough to germinate several per cent. after twenty-four hours immersion. Four parallel lines indicate some germination but very poor development. Three parallel lines indicate fair but distinctly abnormal development, and two lines approximately normal germination and growth.

*Fig. 1* shows in a condensed form the results of some four hundred cultures with fifteen fungi in a decoction of sugar beet to which had been added copper sulfate. A glance at the page will show that many of the fungi present a really remarkable similarity in resistance to copper. Twelve of the fifteen forms represent a range in lethal concentration of from 0.0099 *n* to 0.0168 *n*, or slightly less than 70 per cent. variation. This range of susceptibility may be modified by several important considerations, prominent among which is temperature. All, except Gloeosporium, were grown at 28° C., a little more or less. It is established that a fungus presents its greatest resistance to a deleterious agent, other things being equal, when cultivated at or near its optimum temperature for germination and mycelial development.[8] At a higher or lower temperature a smaller quantity of the agent results fatally. All the forms used, except *Gloeosporium malicorticus*, grew well at 28° C., but it is quite certain that some of them grow better at other temperatures. Inasmuch as the optimum temperatures for the different forms were unknown it was thought best to use a uniform

[8] CLARK, J. F.: On the toxic value of mercuric chlorid and its double salts. Jour. Phys. Chem. 5: 289–316. 1901; also FLENDE: Ueber Creolin und seine Wirksamen Bestandteile. Arch. f. Hyg. 9: ———. 1889.

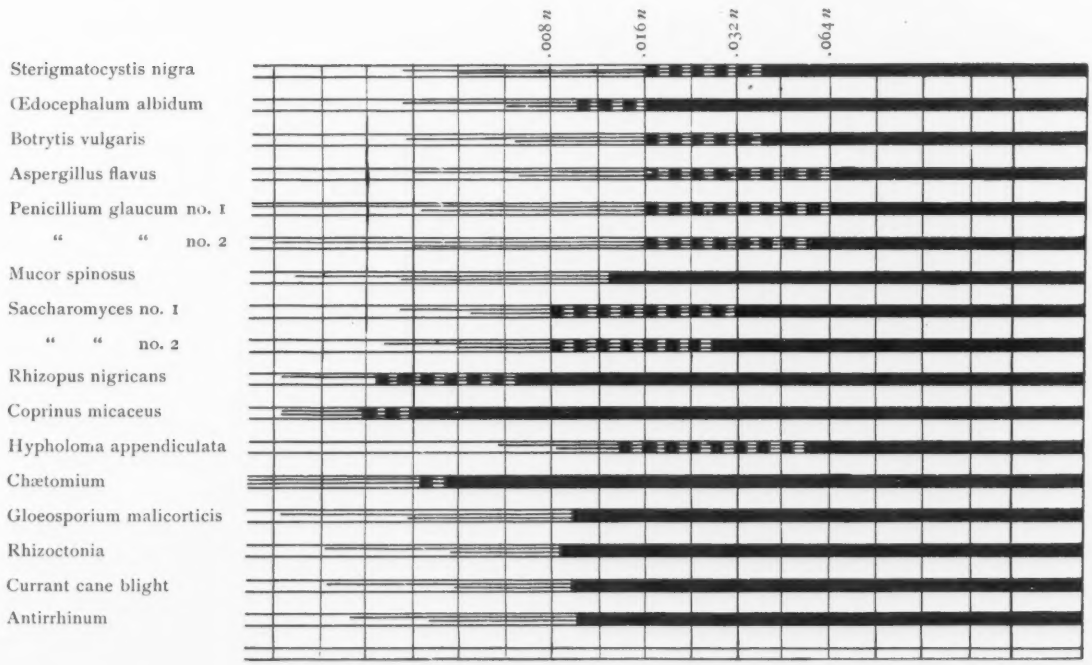



FIG. 1.

NOTE.—On account of the loss of the original drawings, while in the hands of the engravers, it is not possible to give *quantitative* values for this figure. The four values given are approximately correct. *Coprinus* was inhibited by .003 *n*.

temperature, except with Gloeosporium, which refuses to germinate at so high a temperature. It is also doubtless true that other *optimal* considerations have a similar effect in increasing the resistance of plants to unfavorable conditions. It should also be noted in studying these results that several of the forms were tested in media prepared at different times, and as sugar beets vary in composition the media would vary somewhat in nutritive properties, and therefore in toxicological properties also, when used as a solvent for a poison. Making due allowance for these considerations, however, it is perhaps remarkable that of a selection of fungi representing fairly well the natural groups and including typical saprophytic and parasitic forms, the great majority of them should be found to be killed under similar conditions by concentrations of copper of between 0.0099 *n* and 0.0168 *n*, and that the three forms which were found to be markedly more sensitive (Rhizopus, Coprinus, and Chaetomium) should be of such diverse relationships. The two species of yeasts proved to be equally resistant, although markedly different morphologically. The exact similarity of resistance was in this case very easily and prettily shown by growing both forms in the same culture drop. Such a test is applicable only where the forms are of similar properties as regards resistance to the agent, time of germination, and rapidity of growth. Two cultures of *Penicillium glaucum*, obtained from different sources and presenting slightly different habits of growth, also proved to be equally resistant.

The great variation between the two hymenomycetes is partly to be accounted for by the fact that the medium was very favorable for the development of Hypholoma, and only moderately suited for Coprinus. The similarity of resistance of the four parasitic forms (at bottom of figure) is quite striking. It will also be noted that they occupy an intermediate position. The most resistant forms will be seen to be the molds Aspergillus, Sterigmatocystis, Botrytis, and Penicillium.

Dr. Duggar has for some time been studying the broad field of food relation to toxicology in general. It has been

ascertained that fungi vary greatly in their resistance to deleterious agents, according to variations in the medium in which they are cultivated when submitted to the influence of the agent. His results have not yet been published, but the importance of this matter in any toxicological studies necessitates considerable reference to the subject in this study of the toxicology of copper.

The differences in resistance due to variations in the culture media may be due to two distinct causes: (1) All media influence the toxicology of electrolytes, and possibly other agents, by causing certain physical and chemical changes in the condition of the dissolved agent; (2) other things being equal, the more favorable the medium for germination and early mycelial development of a fungus, the more resistant that fungus will be to deleterious agents.

The simplest case of changed physical or chemical conditions would be the diluting of the solution of the copper salt with pure water. Here, with each decrease in concentration, we get less and less of the whole molecule, *e. g.*, $CuSO_4$, and more and more of its component ions, $^{+}Cu^{+}$ and $^{-}SO_4^{-}$, and the amount of change may be readily determined for any particular dilution. When the dilution is accomplished by adding an infusion or decoction of a vegetable or animal substance, however, a series of unknown reactions occurs, and even the amount of copper in the ionic form cannot be known. That these transformations have a very great influence on the toxic properties of the mixture is abundantly shown by the results represented graphically in *fig. 2*. A number of media tested caused precipitation of the copper, thereby rendering it harmless.[9] All such media were discarded and are not represented in the figure.

When spores are placed in a medium which does not admit of growth (*e. g.*, pure water) it is merely a question of time when these spores will be killed, if there be present a sufficient quantity of copper to precipitate the living proteids. It takes some

[9] COPELAND and KAHLENBERG: The influence of the presence of pure metals upon plants. Trans. Wis. Acad. 12: 454–474. 1900.

time, however, perhaps weeks, for all this copper to find its way into the spores. Should the medium be such as admitted growth, the production of protoplasm might easily offset its precipitation by the copper, and long before the copper could have its maximum effect the protoplasm would have increased manifold, and would require a corresponding increase in the concentration of copper to cause death. Thus it is that with every favoring influence the fungus can make a better fight for life.

The results given in *fig. 2*, with various media, and with copper in the form of the sulfate, are those with *Œdocephalum albidum*. The work repeated with *Rhizopus nigricans* gave essentially the same results. A single glance at the figure shows how much more toxic the copper proved to be when dissolved in pure water than when in any other medium, 0.0002 *n* being fatal in twenty-four hours. This is but one-fortieth of the concentration of copper required to give the same result in a decoction of sugar beet. When 1.5 per cent. asparagin was added to this beet infusion the toxic value of copper dissolved in pure water was to its value in the asparagin-beet medium as 142 : 1, and the comparison in the case of the medium compounded from asparagin and inorganic salts the ratio is 285 : 1. In this last case there was a slight precipitate noticed under the microscope, and it may be that some of the copper was precipitated by the phosphoric acid present. Exactly what proportion of this striking difference is to be assigned to the nutritive condition and what to chemical and physical transformations it is impossible to say. Inasmuch as the asparagin medium, while a good food for Œdocephalum, is not so good as beet decoction, we must concede that the remarkable decrease in toxic properties in the cases where asparagin is present is chiefly due to the physical and chemical changes. That these changes have a preponderating influence in the case of the beet decoction is also very probable. The experiment of diluting and concentrating the beet decoction seems to support this view. The values of the triple, the ordinary, the half, and the quarter strengths are not greatly different

.0014n
.0018n
.0024n
.0031n
.0040n
.0051n
.0067n
.0087n
.0011n
.0015n
.0019n
.0025n
.0034n
.0045n
.0058n
.0075n
.0098n
.013n
.017n
.022n
.028n
.036n
.047n
.060n
.079n
CuSO4 in pure water
Decoction of Calla leaves
" " Allamanda leaves
" " pea seeds
" " celery stems
" " Pleurotus ulmarius
" " Lepiota naucena
Beef broth
Infusion of clover hay
Decoction of sugar beet
Beet dec. diluted to two vols. by pure water
" " four " "
" with 1.5% asparagin added
" concentrated to 1/3 volume
Decoction of bean seeds
Pure apple juice
Potato infusion
Prune decoction
MgSO4 .5%; KH2PO4 1%; asparagin 2%; Fe2Cl6 trace

FIG. 2.

so far as stimulation of germination and early mycelial development is concerned. The same is true of the beet decoction to which the asparagin was added. Yet we have the following variation in the lethal concentrations:

| Medium | Lethal concentration | Relative amount of Cu required to inhibit as compared with a pure water solution |
|---|---|---|
| Beet decoction, normal strength..... | 0.0076 *n* | 38 times |
| " " diluted to two volumes..... | 0.0054 *n* | 27 " |
| " " " " four " ..... | 0.0034 *n* | 17 " |
| " " evaporated to ⅓ volume..... | 0.0163 *n* | 82 " |
| " " + 1.5 per cent. asparagin.... | 0.0284 *n* | 142 " |

Were it wholly a matter of food relation we should expect the figures in the third column to be near 38 in each case. Were it wholly a matter of the chemical or physical transformation of the copper by the compounds present in the beet decoctions, we should expect a more proportionate decrease in the first three numbers of the third column, corresponding to the dilution of the medium by the added pure water. As already remarked, it is not possible to determine with any degree of precision the part played by any one factor from the data at hand; but it is evident that with different media great differences in the toxic value of the copper may be obtained, and that asparagin has the power of uniting with salts of copper in such a way as to reduce their toxic properties in a very extraordinary manner. It is of interest in this connection to note that mercuric chlorid is not similarly reduced in toxic properties by the presence of asparagin.[10]

*Fig. 3* presents the results of some studies on the addition of certain compounds to pure water solutions of some copper salts. Here the results given are with Œdocephalum, and, as in *fig. 2*, the results were checked over with *Rhizopus nigricans*. In this diagram the vertical lines representing the variation in the concentration of copper present in the different cultures show an increase of 30 per cent. for each division over the last preceding

[10] Jour. Phys. Chem. 5: 302. 1901.

Copper nitrate in pure water for 24 hours
Copper formate " " "
Copper acetate " " "
Copper chlorid " " "
" " " in 4% $NH_4NO_3$ sol. for 24 hours
" " " " $Na_2SO_4$ (?) " "
" " " " $K_2SO_4$ " "
" " " " KCl " "
Copper sulfate in pure water for 24 hours
" " " 5% grape sugar sol. for 24 hours
" " " 5% cane " "
" " " 4% glycerin " "
" " " 5% $NH_4NO_3$ " "
" " " 5% $Na_2SO_4$ " "
" " " 2% $K_2SO_4$ " "
" " " 5% " "
" " " 5% KCl " "
" " " 3% $K_2SO_4$ for 10 days
" " " 2% pot.-sod.-tartrate for 10 days
.00023n
.00030n
.00039n
.00051n
.00066n
.00086n
.00112n
.00146n
.0019n
.0024n
.0032n
.0041n
.0053n
.0069n
.0087n
.0114n
.0148n
.0192n

FIG. 3.

division passing to the right. It will be observed that the five copper salts, the nitrate, formate, acetate, chlorid, and sulfate, dissolved in pure water have approximately the same toxic value, the variation (0.00004 *n*) being within the limits of experimental error. These five salts are fatal to Œdocephalum in 0.00020–0.00024 *n* concentration in twenty-four hours. This is about a 0.005 per cent. solution of the crystallized salt, $CuSO_4 \cdot 5H_2O$, or one part crystallized salt to 20,000 parts water.

The addition of glycerin, glucose, or cane sugar failed to cause any pronounced decrease in toxic properties. The addition of salts like $K_2SO_4$, KCl, etc., however, caused a very great reduction. One part $CuSO_4$ to 20,000 parts pure water is fatal to Œdocephalum in twenty-four hours. It requires sixty-one parts $CuSO_4$ to 20,000 parts of a 5 per cent. $K_2SO_4$ solution to have the same effect. That the lessening of the toxic value here was not due to a lessening of the rapidity of the penetration of the copper salt is shown by the last three lines of *fig. 3*, where the cultures after standing ten days showed a toxic value of less than one-fortieth that of the $CuSO_4$ in pure water. A test made by adding a small quantity of KCl to solutions of $CuCl_2$ gave an increase in the toxic value of the mixture. Further experimentation on this point is needed, but the indications are that here we have a similar phenomenon to that observed on the addition of chlorids to mercuric chlorid solutions,[11] small additions of a chlorid causing an increase, and larger amounts causing a decrease in toxic properties; the former probably through the formation of highly toxic $^{-}HgCl_4^{-}$ ions, and the latter through the formation of $K_2HgCl_4$ double salt molecules having a low toxic value. Copper reacts very similarly to mercury in respect to the formation of double salts with alkali chlorids.[12] These double salts would naturally be highly dissociated into metallic cations and the anion $^{-}CuCl_4^{-}$, and similar anions. That the ion $^{-}CuCl_4^{-}$ should have a high toxic value is very probable, being in harmony with all at present known regarding toxic

[11] CLARK, J. F.: Jour. Phys. Chem. **5**: 289–316. 1900.

[12] REMSEN, IRA: Inorganic chemistry (advanced course) 589. 1892.

agents capable of uniting with more chlorin.[13] That the forcing back of the ionization of these double salts to the molecular form (*e. g.*, $K_2CuCl_4$) would result in a lowering of its toxic value seems more than probable, in view of what we know in the analogous case of the double salt $K_2HgCl_4$ already referred to, and furnishes a very reasonable hypothesis to account for the remarkable lowering of the toxic value of solutions of copper in pure water when a chlorid is added in excess. The decrease from the adding of sulfates, nitrates, etc. of the alkali bases is doubtless due to similar conditions.

*Fig. 4* gives the results of a test of various soluble and insoluble copper compounds with three fungi grown in beet decoction. The letters *A*, *Œ*, and *B* refer to Aspergillus, Œdocephalum, and Botrytis respectively. Beet decoction has a very marked power of dissolving copper even in the metallic state, hence it was possible to test "insoluble" copper compounds, such as Paris green, copper hydroxid (the form in which the copper is found in Bordeaux mixture), etc. This power of dissolving copper is a property of almost all decoctions of vegetable or animal substances, some possessing it in a much higher degree than others.

*Fig. 4* shows that copper, gram for gram, has much the same value toxicologically when added to a beet decoction, no matter what form of copper salt is used. The apparent exceptions to this are are to be accounted for by the effect of the other atoms and groups added. Thus the chromate salt which proved to be by far the most toxic compound of copper tested owes its high toxic value to the deadly chromium contained.[14] The *eau céleste* and the ammoniacal solution of copper carbonate are particularly fatal to Œdocephalum and Botrytis on account of the basic alkalis present.[15] Œdocephalum is apparently very sensitive to compounds containing arsenic. Perhaps it is especially sensitive to this element, but so far as I know the point has not been tested. That the green arsenoid should be more fatal

[13] DAVENPORT, C. B.: Experimental morphology 8. 1897.

[14] CLARK, J. F.: Bot. Gaz. **28**: 390, 396. 1899. [15] *Ibid.* **28**: 380–382. 1899.

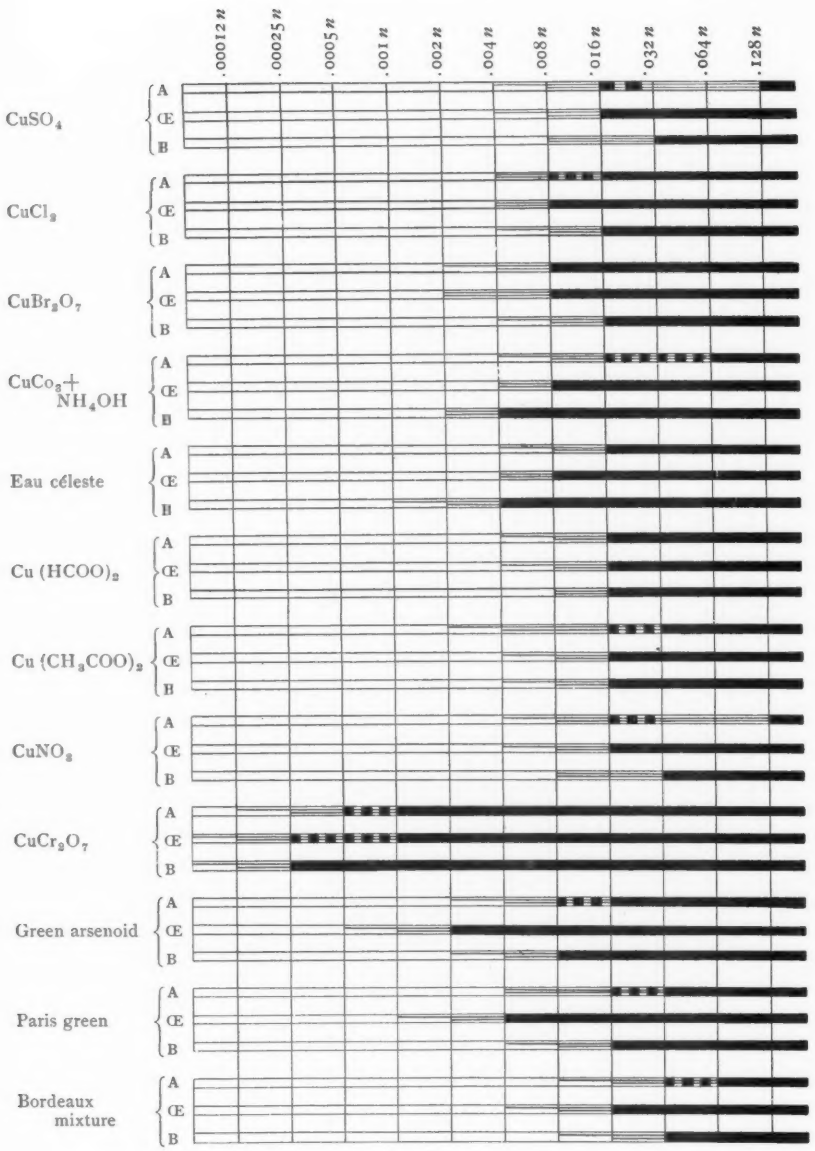

.00012 n
.00025 n
.0005 n
.001 n
.002 n
.004 n
.008 n
.016 n
.032 n
.064 n
.128 n
CuSO4
CuCl2
CuBr2O7
CuCo3 + NH4OH
Eau céleste
Cu (HCOO)2
Cu (CH3COO)2
CuNO3
CuCr2O7
Green arsenoid
Paris green
Bordeaux mixture
A
Œ
B

FIG. 4.

than the Paris green was to be expected, inasmuch as it showed on analysis 8.3 per cent. soluble $As_2O_3$ as compared with 1.8 per cent. soluble in the sample of Paris green. That the ammoniacal solution of $CuCO_3$ is a more effective fungicide than Bordeaux mixture for the amount of copper used does not follow from the showing made in this figure. These tests were made in closed cells where the excess of ammonia was indefinitely retained, whereas under orchard conditions this evaporates in a few hours at most, and exerts practically no fungicidal value.

It would be beyond the province of the present paper to give a detailed account of my experiments on the toxicology of the Bordeaux mixture. These are of greater interest to horticulturists than to botanists. I shall mention briefly, however, the more important experiments and state the conclusions arrived at from this study.

Just how the insoluble copper hydroxid of the Bordeaux mixture sprayed on leaves has protected them so thoroughly from the ravages of parasites has long been a mystery to the chemist and physiologist alike. Various hypotheses have been propounded from time to time, but up to the present no satisfactory explanation has been given. A very careful review of the current hypothesis may be found in Swingle's paper.[16] His own suggestion that it might be possible that the fungi themselves, by secretions or excretions, might be able to aid in the solution of the copper has proved a happy one. As already mentioned, most animal and vegetable decoctions have a greater or less power of dissolving insoluble copper compounds.

This power is particularly noticeable in the fungi. An infusion of *Agaricus campestris*, and infusions and decoctions of various parasitic fungi, dissolve copper hydroxid very quickly and in sufficient quantity to inhibit the germination of any fungus tested. Spores of various fungi placed in a mixture of $Cu(OH)_2$ and water were killed in a short time, while other spores of the same species, placed in similar mixtures of $Fe(OH)_2$

[16] SWINGLE, W. T.: Bordeaux mixture. Bull. no. 9. Div. Veg. Phys. and Path. U. S. Dept. Agric. 1896.

and $Al(OH)_3$ retained their vitality unimpaired for weeks. The death of these spores was not due to the presence of any soluble copper, for no copper could be detected in the filtrate by the most delicate chemical tests before the spores were added. Traces of copper were found some time after the addition of the spores (if added in considerable quantity) showing that they dissolve more of the $Cu(OH)_2$ than was required to kill them. The rapidity with which fungus spores are thus killed naturally varies with the character of the contents and coverings of the spores. It is very quickly accomplished in thin-walled spores, such as Rhizopus and Œdocephalum, but is very slowly accomplished in forms like Aspergillus and Penicillium, whose coats present great resistance to the entrance of poisons and the excretion of copper-dissolving compounds. As an example of such resistance to entrance, see experiments with Aspergillus in solutions of nickel sulfate,[17] in which 0.03 *n* inhibited germination, but immersion in a normal solution (containing over 13 per cent. $NiSO_4$) for 48 hours at 28° C. failed to kill the spores. The same form resists immersion in double normal $ZnSO_4$ (27 per cent. anhydrous $ZnSO_4$) under similar conditions.

The host plant, too, is active in dissolving the $Cu(OH)_2$. A peach tree was sprayed with Bordeaux mixture which contained an excess of lime, as is ordinarily the case. Following this, the tree was sprayed several times during the day with enough pure water to have an effect resembling dew. The following day water collected from the leaves, filtered and evaporated to small volume, showed just a slight copper reaction. Several small leafy branches were then removed and placed in a large beaker of distilled water, without injury to any of the parts. After soaking for some hours these were removed and the solution filtered, evaporated to small volume, and tested for copper. A marked reaction was secured, indicating the presence of considerable copper in solution. The epidermis of leaves, although protected by a cuticle, is well known to be more or less permeable to the dissolved substances occurring in the cell sap, particularly along

[17] CLARK J. F.: Bot. Gaz. **28**: 392. 1899.

the union of the epidermal cells.[18] When the dew is on the leaf we have two solutions—the dew drop without and the cell sap within—separated by a more or less permeable membrane. The result of these conditions must result in the exosmosis of at least some of the contents of the cell sap, which coming in contact with the copper hydroxid adhering to the leaf surface causes more or less of it to pass into solution. An important point in this connection is that the solution of the copper, whether effected by the host or the fungus spore itself, is possible in the presence of an excess of lime. This excess is apparently somewhat detrimental to the solvent action on the copper, and in this way doubtless serves a very valuable purpose in preventing a too rapid solution. It also protects the $Cu(OH)_2$ from the solvent action of the ammonia, nitrites, and nitrates of the atmosphere. According to Millardet and Gayon's[19] experiments, the amount of lime ordinarily used in Bordeaux mixture is sufficient to protect the copper absolutely from solution for eighteen days under ordinary atmospheric conditions. This ought to have been sufficient to have set aside the hypothesis which accounted for the solution of the copper of the Bordeaux mixture by atmospheric conditions, for all experiments go to show that the Bordeaux mixture is effective from the day it is applied. So much in brief review of some of the experiments that have led me to the following conclusions regarding the toxicology of this invaluable fungicide:

1. The solution of that part of the $Cu(OH)_2$ of Bordeaux mixture which under orchard conditions *is of fungicidal value, is chiefly accomplished by the solvent action of the fungus spores themselves*, for they have power to dissolve sufficient copper to kill themselves.

2. The amount of copper necessary for the destruction of the spores of parasitic fungi is probably not more than one part of soluble metallic copper to 80,000 parts water (dew).

[18] BÜSGEN: Ueber einige Eigenschaften der Keimlinge parasitischer Pilze. Bot. Zeit. **57**: 67. 1893.

[19] MILLARDET et GAYON: Les divers procédé de traitements du mildiou par les composés cuivreux. Jour. des Agr. Prat. 1: 701–702. 1887.

3. The host plant has a greater or less power of dissolving the $Cu(OH)_2$ deposited on its leaves.

This solution of the copper by the host plant may or may not be advantageous in protecting it from attack, as injurious spores may or may not chance to be present at the point where solution has taken place. To this factor, however, must be attributed the injury frequently observed with certain plants (*e.g.*, Japanese plums, peach, etc.) when sprayed with Bordeaux. The amount of injury done to a given species will depend on the following considerations, provided always that carefully prepared Bordeaux mixture has been used:

1. The *specific susceptibility* of the protoplasm of the plant to poisoning by copper.

2. The *solvent properties* of the cell sap on copper hydroxid.

3. The *permeability of the epidermis or cuticle*[20] to these cell contents when the conditions are favorable for their exosmosis, and for the entrance of the copper after its solution is effected.

4. Weather conditions following spraying, particularly conditions as regards moisture (dew, etc.), providing the conditions for the exosmosis of some of the contents of the cells of the leaf.

To these might be added, perhaps, climatic conditions which, through modifications of structure, of specific resistance, or of the character of the cell sap, may cause a plant to be more or less resistant to treatment with Bordeaux mixture, according to the locality where it is grown. The very injurious influence of Bordeaux mixture on blossoms[21] is to be explained in an entirely similiar way. Here the protection of the cells by cutinization is almost or entirely wanting, and it is very probable that the rich

[20] Dr. W. C. Sturgis (Rep. Conn. Agric. Exper. Sta. 219–254. 1900) has done some very careful work on the effect of Bordeaux mixture on peach foliage. In his study for a cause of the extreme susceptibility which he found in his peach trees, he has unfortunately overlooked the possibility of specific sensitiveness to copper poisoning of the protoplasm; and in the study of the structure of leaves the study of the cutinization of the epidermis has been omitted. It is hardly possible that the aeration of the spongy parenchyma can be a factor in the case.

[21] BEACH, S. A., and BAILEY, L. H.: Fruit-setting of sprayed blossoms. Bull. 196. N. Y. Agric. Exper. Sta. 1900.

contents of pollen grains and the cells of the stigma have an unusually solvent action on the copper hydroxid.

Swingle's proposition that the copper might be effective in preventing the attack of parasites through negative chemotactic action induced me to make a number of experiments on this point. A number of fungi were used to a greater or less extent,

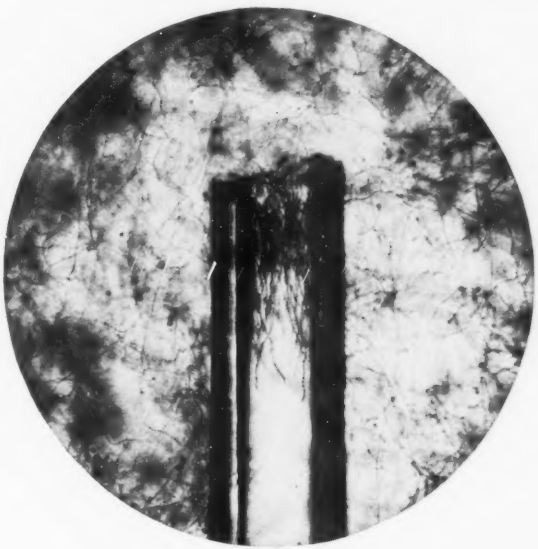


FIG. 5.

but Rhizopus proved the most satisfactory, inasmuch as it usually germinates with a single germ tube and grows rapidly with a minimum of branching.

The well-known method of Pfeffer was first employed. Capillary tubes closed at one end were filled with solutions of copper of varying strengths. Hanging drop cultures of the fungi were prepared in weak agars of varying consistence. Into these hanging drop cultures were introduced the open ends of the copper-containing capillary tubes. These tubes were introduced at different stages of germination. The uniform result of

these preparations was that the hyphae near the mouth of the tube grew into the solution of copper, and continued to grow directly inwards until they reached a concentration sufficient to kill them (*fig. 5*). This was found to be the case with copper, cobalt, nickel, zinc, etc., and *also when the capillary tube contained pure water.*

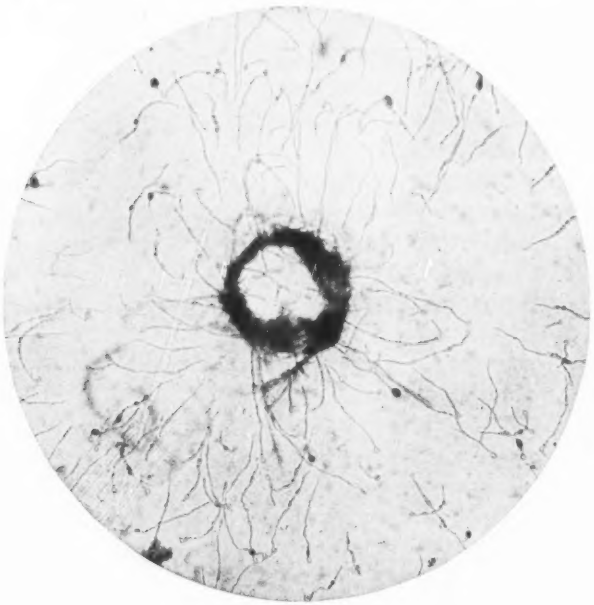


FIG. 6.

Miyoshi's[22] method of injecting leaves with solutions by placing them in the solutions under an air pump and exhausting was next tried. After the injection of the leaves they were washed under the faucet and spores of Rhizopus were sown on the lower surface. The preparation was then placed in a moist chamber in an incubator at 28° C. The germ tubes near stomata curved towards and grew directly into them in a manner quite similar to what Miyoshi found to be the case when the leaf was

[22] MIYOSHI, M.: Ueber Chemotropismus der Pilze. Bot. Zeit. **52**: 1–27. 1894.

injected with food solutions. In many cases upwards of thirty germ tubes were counted growing into a single stoma. On injecting similar leaves with pure water, however, I got a similar result. The leaves used were chiefly from *Tradescantia discolor*. The concentrations of copper, cobalt, etc., injected ranged from 0.01 to 0.005 *n*.

I then tried Miyoshi's method of using perforated mica plates with layers of media between. Spores were sown in every conceivable way: in upper, middle, and lower layers; in nutrient and non-nutrient media; and in media containing copper. The uniform result of these tests was that the germ tubes near the perforations grew towards the opening *if it communicated with a layer of media in which no spores had been placed*. The presence or absence of copper was not found to have any influence on the *direction* of growth. The hyphae grew towards, and in cases where the concentration of copper was not too great, grew through the perforation into the copper-containing medium. *Fig. 6* illustrates such a case. The spores of Rhizopus were sown in a rich nutrient agar. Upon germination they immediately turned towards a perforation in the mica plate which communicated with a non-nutrient agar impregnated with 0.005 *n* $CuSO_4$.

Finally, I prepared a series of cultures in large Van Tieghem cells $5^{cm}$ in diameter. The nutrient agar in which the spores were placed occupied the part marked *a* in *fig. 7*. The portions of agar marked *b b* were prepared from nutrient or non-nutrien agar to which varying concentrations of copper had been added The portions *c c* are checks, being duplicates of *b b* without the copper. The inoculation was made by placing a few spores of Rhizopus at the center of *a*. The cultures were then placed in the incubator at 28° C. until the fungus had made considerable growth. The cells were then opened and *b b* and *c c* were pushed towards *a* until in slight contact and occupying the positions *b' b'* and *c' c'*. More or less of the Cu in solution in *b'* and *b'* would then pass into *a*, and if the fungus was either attracted or repelled by the Cu it would be readily determined by watching

the behavior of the hyphae as they grew down *a* towards the extremities *d d* between the copper-containing portion *b'* and the check preparation *c'*. The hyphae did curve towards *b'* and *b'*, but also towards *c'* and *c'*, and the *larger* the portions *b'* or *c'*, the more strongly they curved towards them. This curving took place even when *b* and *c* contained no nutrient substance, the hyphae growing from the nutrient portion *a* into a nonnutrient medium. Cultures on this plan were repeated many times and varied in many ways, *e. g.*, varying the consistence of the agar, the nutrients used, the concentration of copper, etc.

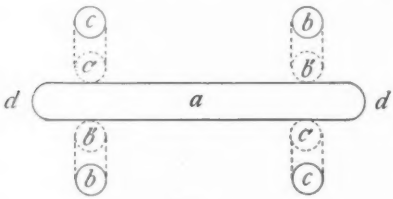


FIG. 7.

My interpretation of these results is that Rhizopus is markedly negatively chemotactic to some secretion of its own mycelium, and this negative chemotropism is much greater than any positive chemotropism it may have for food substances or oxygen. This method then, which seems to be satisfactory, has failed to show that Rhizopus has positive or negative chemotropism towards copper or allied metals. These cultures were used to a less extent with several other saprophytic and two parasitic forms, with the same result in regard to chemotropism towards copper. A large number of photomicrographs and drawings with camera lucida were made, but those reproduced in *figs. 5* and *6* are as instructive as any.

BOTANICAL LABORATORY,
CORNELL UNIVERSITY.

# CLADOCHYTRIUM ALISMATIS.

## CONTRIBUTIONS FROM THE CRYPTOGAMIC LABORATORY OF HARVARD UNIVERSITY. XLIX.

G. P. CLINTON.

(WITH PLATES II–IV)

WHILE collecting at Glacialis pond, Cambridge, Mass., in October 1900, the writer found *Cladochytrium Alismatis* Büsg. on *Alisma Plantago* L. Although the season was so far advanced for this plant that all of the leaves were dead and in many cases broken off, still enough infected ones were obtained to show that the fungus was not uncommon in that locality last year. The discovery of the fungus is especially interesting since this is the first time it has been found in America, and also because the study of it revealed the possession of a peculiar temporary sporangial stage that it was not known to possess.

This Cladochytrium occurs on the leaf blades, petioles, and peduncles, forming lead-colored, generally subcircular sori 1–2$^{mm}$ in diameter. These are generally distinct from one another, and on the woody tissues are apt to have more of an elongated shape. In the leaf blades the so-called spores, or resting sporangia, occupy the cells between the two layers of epidermis, producing a slight pustule which in the green leaves, as shown by *exsiccati* specimens, is surrounded by a rather inconspicuous discolored area. While the sori are long covered by the epidermis, in the dead leaves this eventually breaks open and the sporangia become scattered, thus leaving small circular holes in the parenchyma and pits in the woody tissues.

Although a temporary mycelium is developed in this genus, the fungus in this case was so far matured when discovered that no sign of it could be made out. From one to three or four sporangia were found in the parenchymal cells of the leaf blade (*fig. 33*), while in the more elongated cells of the peduncle there

were often six or more arranged uniserially (*fig. 34*). The sporangia are oval to spherical, with sides sometimes slightly flattened, and vary from 25–45 $\mu$ in longest diameter. The cell wall is reddish-brown and rather thick, the exospore being smooth and prominent, and the endospore thin and hyaline. Attached to the exospore are often the remains of the plant cell. The contents of the sporangium consist of a more or less granular band of protoplasm adhering to the cell wall and a large central oil drop. In sporangia kept indoors for some weeks there seems to be a space between the oil drop and the protoplasmic band, though when young this is entirely occupied by the protoplasm in its more fluid condition.

Fischer (**7**) states that the sporangia of this fungus have never been germinated. The writer was successful in germinating the sporangia abundantly in the many trials made. When first collected a few sporangia would germinate in each trial, after being two or three days in a drop of water; but gradually it took longer and fewer germinated, until after they had been six to eight weeks indoors they ceased to germinate altogether. It was found, however, that if the infected leaves were placed in a small wire cage packed in damp moss and kept in a cool place the sporangia gradually assumed a condition favorable for germination when they were placed in a drop of water. Sporangia thus kept, even at the end of several months, germinated quite abundantly. Material, however, that had been placed outdoors at the same time in stoppered bottles and exposed to the cold of winter, but not to moisture, did not germinate very well. Most of the germinations were made in a drop of water in Van Tieghem cells at the ordinary room temperature.

The germination is similar to that given by Büsgen (**5**) for *Cladochytrium Butomi*. By osmotic action the sporangium becomes swollen until the exospore is broken open. This rupture is regular, a lid being split off in a circumscissile manner from about a third of the sporangium, which begins to crack at one side (*fig. 34*). The endospore meantime protrudes and eventually carries the lid at its top (*fig. 38*), or rarely bends it back as if on a hinge.

The exospore breaks open in so regular a manner that it indicates some definite line of dehiscence, though no such appearance was made out on the mature sporangia before germination. In the meantime there is a change in the contents. So far as could be determined, the large central oil drop is gradually eaten away from its circumference inward until it disappears, or is really broken up into a large number of very small drops scattered through the protoplasm. This latter is now seen to be very faintly divided into areas, showing the formation of the zoospores, in which the small oil drops frequently appear to be arranged more or less peripherally (*fig. 38*). These small oil drops gradually unite in each area to form the final large oil drop of each zoospore (*fig. 39*), and it is these oil drops more than any separating lines that indicate the number and position of the mature zoospores. While this has been going on, the papilla of dehiscence has been forming, though the sporangium is not always placed so that it can be seen. The papilla is best made out when examined in optical section at one side of the sporangium (*fig. 39*). It is usually a somewhat protruding area developed in the wall of the exposed endospore, and begins its dehiscence by the swelling of the cell wall which gradually extends inward as a hyaline area, due to the gelatinization of the wall. In some cases shortly before dehiscence this area seemed to be somewhat pitted, as is mentioned by Büsgen, perhaps allowing a greater flow of water into the sporangium, forming the extended hyaline space in this vicinity. In one sporangium, carefully watched, there could be seen no further change at this point, the hyaline space now having greatly extended inward, until there was a sudden movement of the mass of zoospores, and one coming out into this space darted toward the somewhat distended area in the endospore, and, apparently forcing its way through, escaped, and was followed, one by one by the others, about a hundred, until all had gone. This place of escape usually shows a polygonal opening with a few cracks extending from the angles (*fig. 37*).

The zoospores are somewhat variable in size and vary from

ellipsoidal to spherical in shape. In most cases, however, while active they have the ellipsoidal form. Each one is provided with a prominent oil globule, usually protruding somewhat at one side, and a posterior cilium, three or four times its length, situated at the end nearer the oil globule. A denser mass of protoplasm can be seen on the other side of the oil drop (*fig. 40*). The zoospores are active from the first, moving with a swift-gliding motion for a short distance and then coming to rest, but soon moving on again, so that it is rather difficult to follow one for any length of time. With a high power the cilium can be made out readily when the zoospore is at rest. The movement is always in the direction of the end away from the cilium. The zoospores sometimes apparently remain active for twenty-four hours, and possibly under favorable conditions even longer. Sooner or later, however, they gradually grow more sluggish in their movements, eventually becoming rounded and losing their cilium (*fig. 42*). At this period they often exhibit slight amoeboid movements. They finally come completely to rest and the oil drop gradually disappears. Unless favorably situated they go to pieces, the process in the last stages being assisted by bacteria. A peculiar feature observed at this time was the appearance of a vacuole in which could be seen one or two irregular somewhat elongated protoplasmic bodies that kept up a constant vibration which did not seem to be merely Brownian motion (*fig. 43*).

After it was found that the resting sporangia of this fungus would germinate, it was thought desirable to see if further information concerning its life history could be made out. Accordingly some seeds of *Alisma Plantago* L. were gathered and germinated. This germination took place very slowly, requiring a preliminary soaking of the seeds in water for three or four weeks. The young seedlings first send out a filiform cotyledon, and the succeeding leaves for some time are merely phyllodia adapted for aquatic existence. These have chlorophyllous epidermal cells, but are so thin that they can be watched easily even under high powers of the microscope. In

some of the experiments the plants were allowed to take root in soil covered with water, and the leaves removed as desired for examination. It was found, however, that even a piece of a leaf would remain fresh and active in a drop of water in a Van Tieghem cell for several days, and thus could be watched directly. The best results were obtained with young seedlings whose roots were placed in one of the arms of a Ward cell partly filled with a nutrient solution, and the first grass-like leaf was held up by a little cotton support so that it adhered to the cover glass containing a drop of water and the sporangia.

This species of Cladochytrium is placed by some botanists under the genus Physoderma, because it was not known to have a temporary sporangium. Our experiments were somewhat surprising, since they showed not only that such a stage existed, but that it was of a quite peculiar type. When this was first seen its relation to Cladochytrium was not suspected, but when it became evident that the two were connected, a large number of culture experiments were made in order to remove all doubt. The result was that this temporary stage appeared in every case where the resting sporangia germinated in water containing the young Alisma, and failed to appear in every case where the sporangia did not germinate and in the checks, both where nothing was placed with the young Alisma and where merely a piece of the old Alisma leaf which contained no sporangia was added.

As the zoospores are usually quite active for some time, it was impossible to follow a single one from the time of its exit from the sporangium until it came to rest. It was not difficult, however, when they began to grow sluggish in their movements, to find them settling down on the Alisma leaf and to watch them in their subsequent development, so that there was no doubt as to their identity. They almost always fix themselves on the epidermis just above the wall dividing two cells (*figs. 1–4*). Many do not succeed in penetrating the leaf, as the majority of those that were continuously observed during this stage failed to effect an entrance. In such cases the zoospores are sooner or

later destroyed. In other cases, however, and usually within twenty-four hours after settling down permanently, a short rhizoid (*fig. 3b*) can be seen penetrating the cell. In the meantime the cilium has disappeared, and the oil drop has been growing smaller, being used apparently for the development of the rhizoid. This becomes somewhat club-shaped and soon develops from the swollen tip short branches which eventually grow out into slender branched and somewhat irregular threads (*figs. 16–18*). By means of this rhizoid the young sporangium, as it may now be called, gains nourishment and soon begins to increase in size, so that by the end of the first day after its penetration it may have grown to twice its former diameter. The chlorophyll grains of the host, which normally are distributed rather evenly over the cell wall, are now seen to be congregating in the vicinity of the rhizoid, so that very often all of them are eventually clustered there (*fig. 4*). Sooner or later the young sporangium ceases to swell uniformly and becomes constricted at places, so that it develops a number of folds. These give the elongated forms a somewhat bilateral, and the circular forms more of a radial symmetry. The sporangia keep on enlarging until sometimes they reach over 80 $\mu$ in diameter, though often they may increase but little beyond the size of the original zoospores. This variation is determined by condition of leaf, number of competing sporangia, etc. Usually the rhizoid is not extensively developed in the cell, so that after the sporangium begins to grow it is hidden from view.

After the temporary sporangium has attained its full size, its protoplasm becomes faintly divided into areas, about the circumference of which the numerous small oil drops seem to have chiefly congregated (*fig. 6*). In time the small oil drops of each area unite into the large drops of the individual zoospores, and it is by these, rather than by the appearance of visible boundaries, that the zoospores may be distinguished. While the zoospores have been forming, the papilla of dehiscence has been developed at one end or side of the sporangium. There is usually a space here free from zoospores, and the cell wall by

gelatinization forms a distinct hyaline area extending inward in a manner similar to that already described in connection with the germination of the resting sporangia (*fig. 12*). At the time of dehiscence this gelatinized envelope is often protruded at the opening. Into this vesicle the zoospores dart, forming a ball, though it becomes dissolved and the zoospores scattered before all have escaped. The number of the zoospores that is produced depends on the size of the sporangium and varies from three or four up to probably as many hundred. The zoospores are like those formed in the resting sporangia except that they are possibly somewhat smaller. They settle down on the leaf in time, and give rise to secondary temporary sporangia, and even a third generation has been observed on the same leaf. Because of their crowded condition, the failing power of the leaf to furnish nourishment and its diseased condition, these second and third generations, at least in cell cultures, often produced sporangia of smaller size, and their zoospores seemed less active, in extreme cases even failing to move around after being discharged. Sometimes such zoospores had oil drops of a sickly reddish-yellow color. In leaves kept in cultures for some time, occasionally there occurred areas in which the sporangia covered half the surface of the leaf (*fig. 20*).

After a sporangium has discharged its zoospores, if the conditions for growth have been favorable, it is seen that a second sporangium has started within. The rhizoidal apparatus is separated from the sporangium by a septum, which, as soon as the zoospores have escaped, becomes protruded as a papilla, by the further growth of which the second sporangium is formed (*fig. 7*). This process may be repeated until in some few cases as many as five or six sporangia have been developed concentrically. Usually only one or two additional ones are formed, or even only the original one (*fig. 17*). They all dehisce at the same point, though this place of discharge often becomes obscured (*fig. 17*). The successive stages in the development of a temporary sporangium from a given zoospore derived from a resting sporangium are shown in *b*, *figs. 2–9*. The similar development of

two small sporangia from the zoospores of a temporary sporangium is shown in *figs. 10–15*.

In a few cases, about three weeks after the first infection by the zoospores, there were also found mature resting sporangia similar to those found in the old leaves in the autumn (*fig. 46*). In one case the first sign of these was seen about two weeks after the first infection.

The development of the resting sporangia is quite similar to that described by Büsgen for *Cladochytrium Butomi*. The zoospores penetrate into the leaf much as they do for the formation of temporary sporangia, but in this case all of the contents of the zoospore passes into the enlarged end of the rhizoid-like process. This enlarges into a greatly swollen cell that eventually forms the *Sammelzellen* of authors (*fig. 25*). After this cell has reached its full size, a cell is separated by a partition across the smaller basal end (the end connected with the penetrating zoospore), which is less rich in oil globules than the larger apical or free end. This latter cell may also become divided into two or more, apparently sometimes temporary compartments. From this apical cell or cells are developed one to several very fine simple mycelial threads which connect the *Sammelzellen* to the cell walls of its host (*fig. 29*). On one side of the basal cell near the septum a rhizoid-like process is usually seen. In the same vicinity, evidently connected with this basal cell (though this was not surely made out) and more or less embraced by the rhizoid-like process, there appears a small round cell that rapidly enlarges into the sporangium, while the basal cell becomes empty of its contents (*figs. 27–29*). One or more of the mycelial threads may penetrate into another cell, and there give rise to a similar apparatus (*figs. 28–31*). In this way the fungus spreads from cell to cell. About this time the apical portion of the *Sammelzellen* becomes more or less collapsed (*fig. 29*), but whether this is due to the production of the secondary *Sammelzellen* developed from it, or to a process of fertilization, as claimed by some botanists, was not determined. On the side of the young sporangium there is also often seen a

rhizoid-like process. The exact nature of these processes is not clearly shown, though very often they seem to bind the sporangial cell to the *Sammelzellen*. The sporangium while young contains numerous small oil globules scattered through the protoplasm, but these gradually unite into the one central oil drop. The sporangial walls also gradually thicken, become tinted, and the sporangium is finally matured (*fig. 46*). In the young sporangia there is some indication of a special place for the dehiscence of the cap. Young sporangia that were slightly crushed under a cover glass split off a cap on the side away from the *Sammelzellen*. This is evidently the side that in the mature sporangia is occasionally seen somewhat cupped. Mature sporangia, however, when crushed, do not always split open so regularly.

The infection experiments were all made with the aquatic leaves of seedling plants. It seems very likely that the nature of the leaves determines the character of the sporangia formed, the exposed temporary sporangia being adapted to the peculiar aquatic leaves, and the internal resting sporangia to the aerial leaves. There were some indications that the temporary sporangia did not develop so abundantly on the later leaves, while in none of the aquatic leaves did the mature sporangia develop to such an extent as one might expect if these presented the most favorable conditions for their production. What different conditions determined, as was sometimes the case, the production of both stages on the same leaf was not discovered, but a possible explanation may be that the resting sporangia were developed from zoospores that after settling on the leaf were exposed to the air.

The time relations in the development of the different stages of this fungus are shown approximately by the following observations made in one case:

March 21. Resting sporangia placed in water with young Alisma leaf.

March 23. Sporangia beginning to split off caps.

March 23–24. Active and quiet zoospores present.

March 25. Some zoospores have penetrated leaf to form young temporary sporangia.

March 26. Temporary sporangia of larger size.

March 28. Many temporary sporangia emptied of zoospores.

March 30. Oldest temporary sporangia emptied a second time; second crop of temporary sporangia developing.

April 1. Second crop of sporangia matured; third crop developing; signs of resting sporangia.

April 8. Several mature resting sporangia found.

Despite the luxuriant production of zoospores there are a number of causes that may prevent their forming sporangia. In the first place, they seem to be in their fresh condition a coveted prey for a number of the lower animals. In cell cultures amoebae were seen that had devoured eight or more. Even some of the little heliozoa, scarcely larger than a zoospore, were seen to catch as many as six before they became apparently satisfied. But the most ravenous enemies were occasional rotifers, which did not seem satisfied with a capture of several hundred. In the second place, the zoospores must reach their host and settle down upon it. Very likely many fail to do this. Finally, after reaching the host there are those that never succeed in penetrating into the tissues.

The resemblance of the temporary sporangia to the forms described by Zopf as *Rhizophyton*, on desmids and unicellular algae, suggested the possibility of the temporary sporangia developing on such hosts. In no case, however, where germinating resting sporangia were placed with various species of algae and desmids, were there any indications that the zoospores tried to penetrate them.

The discovery of the temporary sporangia for this Cladochytrium suggests the possibility that other members of the Physoderma section of the genus may also have a similar stage. Through the kindness of Professor Thaxter the writer succeeded in obtaining leaves of *Menyanthes trifoliata* infected with *Cladochytrium Menyanthis*. These leaves had been out doors all winter, and were badly rotted when obtained. The resting sporangia, however, germinated rather readily when placed in water. This Cladochytrium differs from the one on Alisma in the smaller, lighter-colored resting sporangia, which are also

more permanently bound together by the cells of the host. Apparently it has also a very thin outer wall. Its germination differs decidedly in that no cap is split off. Instead, an elongation takes place at one end, as if the inner wall had pushed through the outer wall and the encumbering plant tissue. At the end of this prominent outgrowth is produced the papilla of dehiscence, and the subsequent history is similar to that of the other species (*figs. 21–24*). Attempts to infect young Alisma leaves and various algae with the zoospores from this species were not successful. Unfortunately, seeds or plants of the Menyanthes were not at hand for infection experiments.

*Cladochytrium Alismatis* was first described in 1833 in Germany by Wallroth (**1**), who named it *Physoderma maculare*. Its identity has since been fully established, as the original specimens of Wallroth were examined and figured by De Bary (**2**) in 1864. Fuckel (**3**) in 1869 transferred the fungus to the genus Protomyces, to which it evidently does not belong. In 1887 Büsgen (**5**) placed it in the genus Cladochytrium, and also changed the specific name to *Alismatis*. There was no apparent reason for this latter change, unless it was to make a uniform usage in his paper of having all the species named after their hosts. Strict adherence to priority would require the use of Wallroth's rather than of Büsgen's specific name. Schroeter (**4, 8**) and Berlese and De Toni placed the fungus under Physoderma, Schroeter distinguishing this from Cladochytrium by the absence of temporary sporangia. Fischer (**7**), on the other hand, does not consider them distinct, and places them with Schroeter's genus Urophlyctis under Cladochytrium.

The fungus has been found only on *Alisma Plantago* L. and its varieties. It is reported in Saccardo's *Sylloge Fungorum* as occurring in Germany, Finland, France, Italy, and Siberia. The following *exsiccati*, which have been examined by the writer, contain specimens: Krieger, Fungi saxonici, 681, *Physoderma maculare;* Rabenh.-Wint.-Paz., Fungi europaei, 3977, *Physoderma maculare;* Sydow, Phycomyceten et Protomyceten, 45, *Cladochytrium Alismatis;* Sydow, Mycotheca Marchica, 4331,

*Cladochytrium Alismatis;* SYDOW, Phycomyceten et Protomyceten, 141, *Physoderma maculare.* Numbers 4867 of ROUMEGUÈRE'S Fungi selecti exsiccati, 1417 of DE THÜMEN'S Mycotheca universalis, and 1 of KARSTEN'S Fungi Fenniæ exsiccati, though thus named, are specimens of *Doassansia Alismatis.*

The writer wishes to acknowledge his indebtedness to Professor Thaxter for very helpful assistance in the study of this fungus, and to Professor Farlow for some of the references to the literature and the opportunity to examine herbarium material.

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## EXPLANATION OF PLATES II–IV.

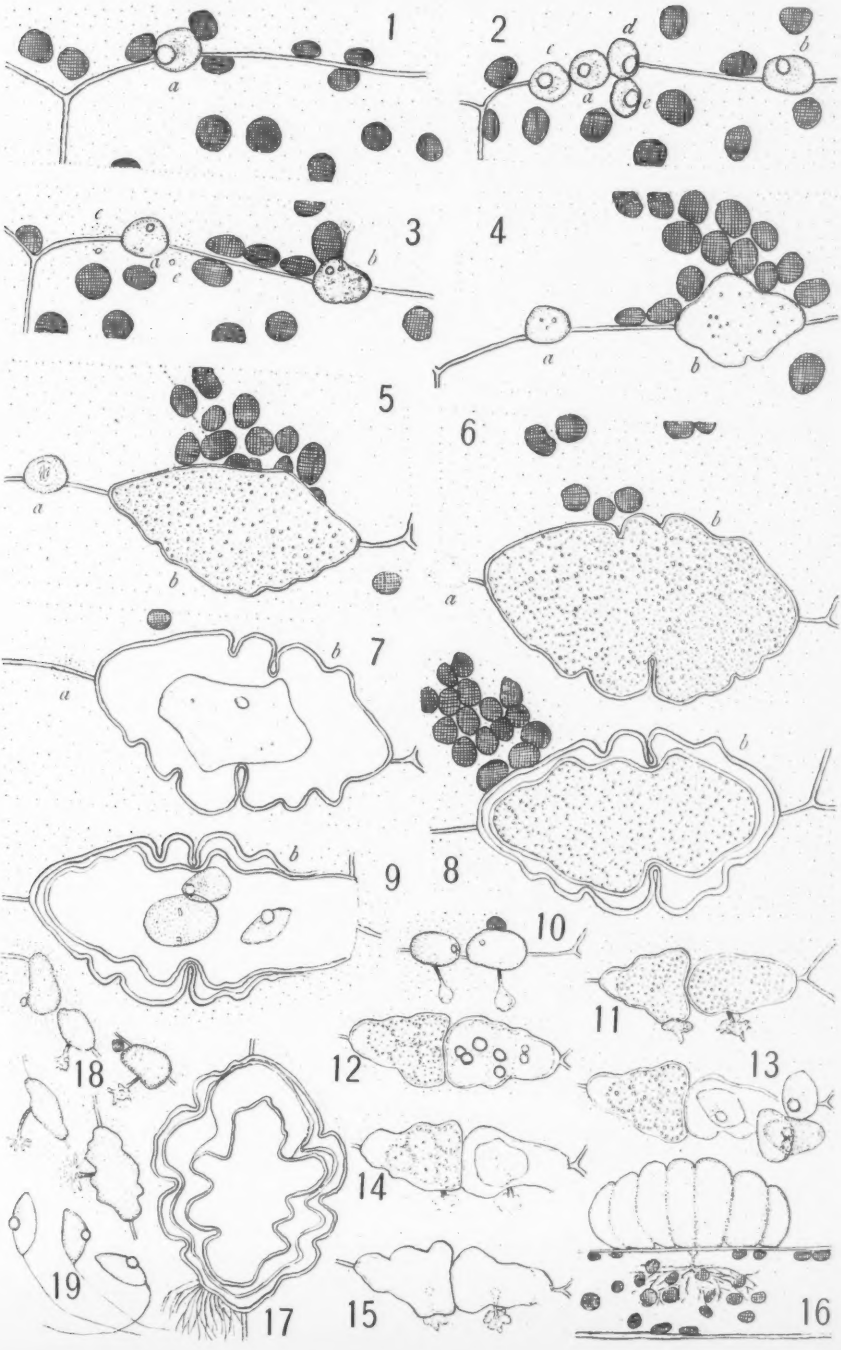
All figures magnified about 850 diameters, excepting *16, 17, 33–39* (about 550 diameters), and *20* (about 300 diameters).

*PLATE II.*

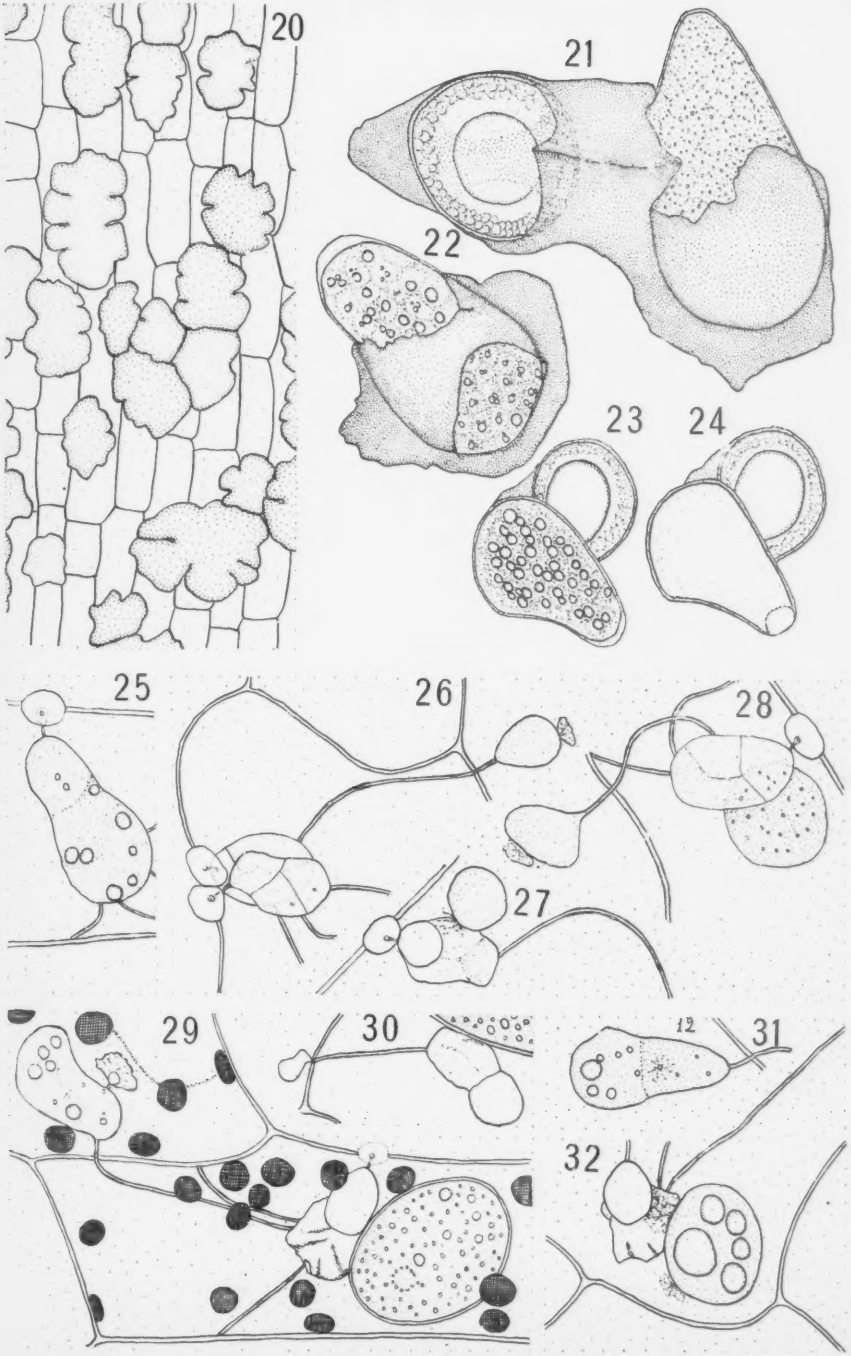
FIGS. 1–20. Temporary sporangia of *Cladochytrium Alismatis.*

FIGS. 1–9. Stages in the development of a temporary sporangium, produced from a zoospore of a resting sporangium, during nine consecutive days.

FIGS. 10–15. Stages in the development of two small temporary sporangia, produced from zoospores of a temporary sporangium, during three successive days.




CLINTON on CLADOCHYTRIUM

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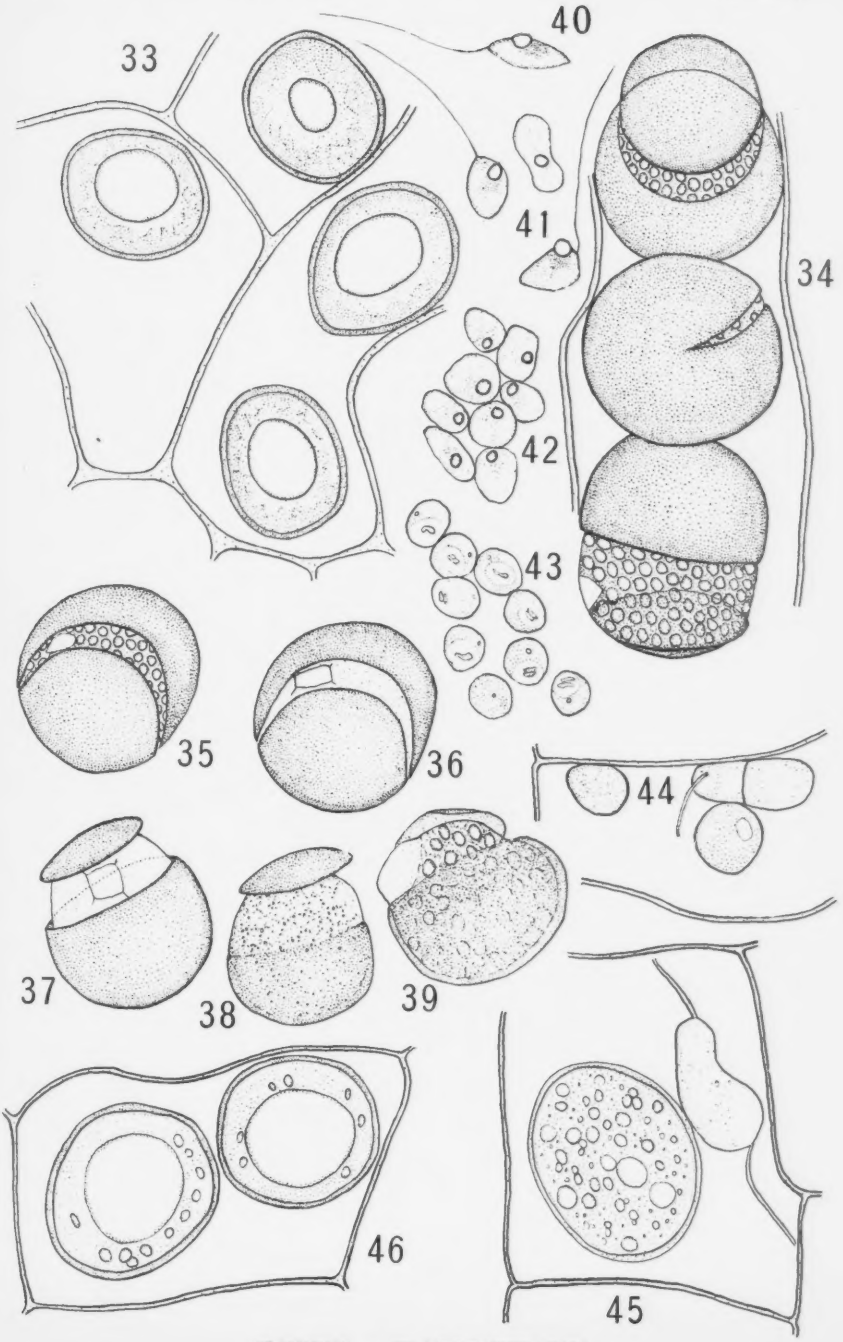


CLINTON on CLADOCHYTRIUM

FIG. 16. Side view of a temporary sporangium showing its rhizoid.

FIG. 17. A temporary sporangium that had emptied three times.

FIG. 18. Several young temporary sporangia, illustrating the early development of the rhizoid.

FIG. 19. Zoospores from temporary sporangium.

*PLATE III.*

FIG. 20. Epidermal view of portion of Alisma leaf showing numerous immature temporary sporangia.

FIGS. 21–24. Successive stages in the germination of *C. Menyanthis*, the sporangia being bound together by remains of plant cells.

FIGS. 25–32. Stages in the development of the resting sporangia of *C. Alismatis*.

FIG. 25. Penetrating zoospore with *Sammelzellen* and mycelial threads.

FIG. 26. Two penetrating zoospores with *Sammelzellen* and mycelial threads, one of which is forming a young *Sammelzellen* in an adjacent cell.

FIG. 27. *Sammelzellen* with attached young sporangial cell.

FIG. 28. Sporangial cell further advanced.

FIG. 29. *Sammelzellen* collapsing, sporangium about full size, and secondary *Sammelzellen* well advanced.

FIG. 30. The beginning of secondary *Sammelzellen*.

FIG. 31. Secondary *Sammelzellen* dividing into apical and basal cells.

FIG. 32. Secondary *Sammelzellen* collapsing, with attached young sporangium.

*PLATE IV.*

FIGS. 33–46. Resting sporangia of *C. Alismatis*.

FIG. 33. Section through cells of old leaf of Alisma, showing resting sporangia

FIG. 34. Germinating sporangia still imbedded in woody cell of petiole.

FIGS. 35, 36. The same resting sporangium just before and after emptying its zoospores.

FIG. 37. Side view of an empty resting sporangium.

FIG. 38. Resting sporangium with protoplasmic contents divided into areas, small oil drops, and beginning of papilla of dehiscence.

FIG. 39. Resting sporangium with prominent lateral papilla of dehiscence, about to discharge its zoospores.

FIG. 40. Fresh zoospore from resting sporangium.

FIG. 41. Older and sluggish zoospores.

FIG. 42. Still older zoospores, having lost their cilia.

FIG. 43. Old zoospores going to pieces, showing vacuoles with peculiar vibrating bodies.

FIGS. 44–46. Stages of resting sporangia in cells of young aquatic leaves of Alisma; produced by artificial infection.

# BRIEFER ARTICLES.

## CLUES TO RELATIONSHIP AMONG HETEROECIOUS PLANT RUSTS.[1]

THE collection and study of Uredineae are beset with a difficulty not found in connection with other classes of plants. A large number of species, principally belonging to the genera Puccinia and Uromyces, inhabit two wholly diverse species of host, and the form of the fungus assumed upon one host gives no clue to the alternate form upon the other kind of host. If teleutospores, for instance, are found upon a grass, there can be nothing detected in the morphology of the rust or of its host, with possible rare exceptions, to indicate whether the corresponding aecidiospores are to be looked for upon a monocot or a dicot, upon a liliaceous, ranunculaceous, or composite plant, or plant of some other family, upon a tree, shrub, or herb; in fact there is no clue whatever. So it comes about that the two halves of heteroecious species are usually gathered separately and listed independently under different genera.

Probably most localities will furnish from a dozen to twenty-five species of aecidia, and *per contra* the same number of unattached teleutosporic forms. Or, to use the prevailing method of expression, there will be so many heteroecious species showing teleutospores, and an equal number of undistributed aecidia. Yet it stands to reason that whether the aecidial or the teleutosporic half be found, still it is but half the species. Contrary to general opinion, there is no definite proof that the one half has any more physiological or taxonomic importance than the other, and the small consideration at present given by collectors and students to the aecidial forms is unfortunate and unwarranted.

For some time I have carried on successful cultures with the heterœcious Uredineae, by which spores of one half of a species were sown, and eventually gave rise to the spores of the other half of the same species. To accomplish this it is necessary to have a pretty clear clue to

[1] Read before the Botanical Socicty of America, Denver meeting, August 1901.

the host on which the second half grows. For instance, if I have teleutospores, found upon a grass in germinating condition, shall I sow them upon a geranium, gooseberry, buckeye, thistle, or some other one of a hundred or more plants known to bear aecidia? I might try each one in succession, and hope eventually to hit upon the right one. But the chances of success by this method are few, in fact, according to my experience, far too few to warrant its use. On the contrary, in order to cherish any reasonable hopes of success it is necessary to possess very definite clues. The method of obtaining these clues I desire especially to call attention to, for it seems to me that observation of this kind will add at times much interest to collecting, and provide valuable data to supplement that usually taken.

To make the problem more concrete, I may state that during the present season I have grown from teleutosporic material the common aecidia on Sambucus, Erigeron, and Aster, and an aecidium on Ribes that appears to be different from the common form. The teleutospores for these four species of rusts came from as many different species of Carex, and have heretofore been erroneously listed as *Puccinia Caricis*. The taxonomic significance of these results will be brought out when the data are ready for publication; it is now only intended to be shown how I came to sow these particular teleutospores upon the right hosts.

In the first place, all observations affording clues (with such exceptions as the similarity in form between uredospores and aecidiospores to be detected only with the microscope, which are so rare as to be negligible) must be made in the field. For the most part they must be made previous to the season when the cultures are undertaken. It is advantageous, indeed, to watch the same rusted plants, or clumps and areas of rusted plants, from season to season. In this way the interest is not so much centered in the specimens carried away, as in the fungus crop that is left behind. When specimens are gathered, they are labeled not only with the usual data, but with sufficiently exact directions so that the spot may be found again. The same host plants are then examined from time to time as the succession of spore-forms appears — spermogonia, aecidia, uredo, amphispores, and teleutospores, all or in part as the case may be. It is like watching for a succession of blooms, and the pleasure of collecting is much enhanced. The whole available territory over which one may ramble or drive now becomes mapped out into specific localities where this, that, or the

other kind of rust grows, and what was mere collecting has resolved itself into an ecological study of absorbing interest.

Only a rich and plentiful occurrence of the rust, whether over wide or restricted areas, is of much value to furnish a clue to relationship. Scattering aecidia or teleutospores generally mean that the source of infection is at a considerable distance, and what is found has come from chance spores blown long distances by the wind. Species vary much as to the distance spores may be carried in condition to start infection, but for our present purpose any distance from a hundred feet to a mile or more is likely to be enough to render our deductions very uncertain.

Probably the easiest and clearest clue is obtained by watching for the first appearance of aecidia. If they can be found thickly covering the leaves of a vigorous plant, especially near the ground, and very few or no aecidia of the same kind upon adjoining plants of the same species, let us search carefully underneath for dead leaves of grass or sedge bearing teleutospores, and for a foot or two around. If these can be found, a little additional search will generally bring to light fragments of last year's inflorescence, by which the specific identity of the teleutosporic host may be determined. The leaves closest to the rusted grass or sedge, which is presumably the source of infection, should bear the thickest sprinkling of aecidial spots. If now a careful search reveals within a radius of ten or twenty feet, only this one grass or sedge bearing teleutospores, we have good reason to assume that the aecidia have arisen from sporidia derived from the recent germination of the teleutospores, and that the two are parts of the same species. By continuing our observations we ought to find after a time, if the weather is propitious, that uredosori are appearing upon the green grass (or sedge) leaves, more abundantly upon those close to the aecidia, and fewer the greater the distance. Only the earliest sori can be of value for our purpose, because uredospores germinate readily, as a rule, and give rise to uredosori that cannot be distinguished from those arising from aecidiospores. If such juxtaposition of the two kinds of spore formation, such coinciding of two centers of infection, be found elsewhere, or for more than one season, especially when other sorts do not occur in a way to cause confusion, we are provided with a strong clue to relationship. Of course, nothing short of cultures will enable one to be perfectly certain. A clue of this sort led to the right sowing for the Aster aecidium mentioned above.

Such good fortune as finding the two halves of a rust so clearly

associated does not occur frequently, although more often than the uninitiated might suppose. A clue of less value may be obtained by finding a limited area monopolized by a single species of grass or sedge, the whole well rusted. We now search for aecidia upon herbs, shrubs, or trees growing within the rusted area, or not to exceed a hundred feet from it. Abundance and nearness, and taking into account all other forms, give us our clue. This was the kind of clue used for the sowing of Sambucus. A Carex of very distinctive habit, species unknown as the plants were sterile, grew in a thick mass, not over ten feet across, that was heavily rusted each season. This spot had been under observation a number of years. No other locality in the immediate region was known for the Carex, and owing to the character of the surrounding ground, it could scarcely find a foothold within a half mile, or possibly much more. No aecidia appeared on any plant growing among the Carex, or within ten feet of it. It was a rich region for plant rusts, and within one hundred feet aecidia were found upon Ranunculus, Oenothera, Impatiens, Eupatorium, Napaea, Sambucus, Ptelea, and Urtica. The aecidia of Ptelea and Urtica were known to belong to other teleutospores ; the aecidia on Ranunculus and Oenothera were too abundant and widely diffused through the adjoining region to warrant their being considered. This left four kinds of aecidia sufficiently local to be accepted for trial, and after two season's work it has been positively ascertained that this particular Carex rust must belong to the aecidium on Sambucus.

When a rust occurs very sparingly in a region, but richly developed, or when it is restricted to a rare host, close watch in the immediate vicinity is sometimes rewarded by the discovery of the alternate form. Occasionally this alternate form is so conspicuous that it has been previously collected, but not infrequently it proves to be a sort not before recorded for the district.

The better one becomes acquainted with the rusts of a small region, the more chances there are that his inferences regarding relationship will stand the test of cultures. If he does not make cultures himself, some one may be found who will be willing to undertake the sowings, provided material be furnished. This consists of small living plants of the supposed host of the aecidia, and grass or sedge leaves bearing the rust, the latter gathered during the previous winter and subsequently kept out of doors. Both are readily sent by mail at a trifling cost. Cultures with use of aecidiospores are also important, but less

easily arranged for when they are to be made at a distance from the source of material. Sometimes a single sowing of teleutospores determines the point in question, but for a variety of reasons it usually does not. A common source of difficulty is the failure of the teleutospores to germinate. But these are details pertaining to a separate matter. It will be many years before any large proportion of our numerous heteroecious rusts will be connected with their respective aecidia, and in the meantime all clues to relationship will be much prized by students, and their pursuit will give to the collector an additional source of pleasure.—J. C. ARTHUR, *Purdue University*.

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## ROCKY MOUNTAIN PLANT STUDIES. I.

HAVING spent the past two collecting seasons in the field with Professor Nelson, and having had the opportunity of much observation and some investigation in the herbarium, I have become greatly interested in the varied and beautiful flora of the middle West. No one who comes into close contact with the plants of this region can fail to wish to know more of them. It is my belief that the following plants of this region have so far remained unrecognized. The types are all deposited in the Rocky mountain herbarium of the University of Wyoming.

**Marsilia oligospora,** n. sp.— Plant 4–7$^{cm}$ high: leaflets woolly or becoming glabrous, 6–10$^{mm}$ long, 3–7$^{mm}$ wide: sporocarp solitary, 4–6$^{mm}$ long, 4–5$^{mm}$ wide, covered with long straight and appressed (rarely somewhat woolly) pubescence: raphe short: lower tooth short and blunt, upper a mere rounded papilla or wanting: peduncle 5–8$^{mm}$ long: sori 5–8 in each valve: megaspores oval to barely oblong, 6–9 in each sorus.

This is a species which has passed for *M. vestita*, but seemingly bears no very close relationship to it. The absence of the sharp upper tooth on the sporocarp, which is so prominent in *M. vestita*, is a mark by which they may readily be distinguished in the field. The number of sori in each valve is less in *M. oligospora* than in *M. vestita*—5 to 8 in former, often 10 or 11 in the latter. The number of megaspores in the two is noticeably different, each sorus in *M. vestita* containing at least 12 and usually 18 to 20. There is also quite a noticeable difference in the shape of the megaspores and in the character of the pubescence of the sporocarp.

The type is number 6560 by *Aven Nelson* and *Elias Nelson*, from Jackson's

hole, Uinta co., Wyoming. It occurred in the bed of a drying-up marshy lake.

**Erythronium obtusatum,** n. sp.— Bulb rather thick, 3–5$^{cm}$ long, deeply imbedded in the soil: leaves oblong-elliptical, 10–20$^{cm}$ long, 3–5$^{cm}$ wide, obtuse but usually apiculate, never mottled: scape stout, usually 2–3-flowered (1–5): perianth segments pale yellow, drying with a purple tinge (in the field drying white), 2.5–3.5$^{cm}$ long, 6–15$^{mm}$ wide, usually reflexed: filaments dilated at the base and tapering gradually to a point at the anther; anthers 5–8$^{mm}$ long, dark purple: stigma deeply 3-lobed; style very slender, usually equaling or exceeding the stamens.

This species is most closely related to *E. grandiflorum*, from which it differs in its very broad, obtuse leaves, its pale yellow and purple-tinged (at least when dry) flowers, its shorter, dark-purple anthers, and its dilated filaments. It probably has also been referred to *E. Hendersoni*, but this is out of the question since that calls for mottled leaves, purple perianth segments, fleshy subacute auricles at the base of the inner perianth segments, the two scales subglobose-inflated, very slender attenuate filaments, and a shortly 3-lobed stigma (ours deeply 3-lobed), none of which characters *E. obtusatum* possesses. It is barely possible that, in large part, the Erythroniums of Montana, as well as those of northern Wyoming and adjacent Idaho, are to be included in this species.

Two collections of it are at hand: Yellowstone park, Glen creek, 1889, *Aven Nelson* and *Elias Nelson*, no. 5606 (type); Idaho, Continental divide. 1899, same collectors, no. 5480 (co-type).

**Erythronium parviflorum** (Wats.).—A low plant with opposite leaves: bulb slender, 3–5$^{cm}$ long: scape slender, 10–20$^{cm}$ long: leaves oblong, tapering gradually to acuteness at both ends, 10–15$^{cm}$ long, 3–4$^{cm}$ wide: flowers rarely more than one, the peduncles abruptly curved in the form of a shepherd's crook: perianth segments broadly lanceolate-acuminate, 2–3$^{cm}$ long, about 1$^{cm}$ wide, bright yellow with a very pale greenish base, strongly reflexed: filaments slender, 1–1.5$^{cm}$ long; anthers short, a little lighter than the petals: style clavate: ovary and capsule broadly oblong to even oval, 2–3$^{cm}$ long.

This species differs from *E. grandiflorum* principally in being much smaller, in its smaller bright yellow flowers with light colored centers, its abruptly curved peduncles, and its very short anthers. The description of *E. grandiflorum parviflorum* Wats. is very indefinite and incomplete, but undoubtly refers to the above form, which is well worthy of specific rank.

Wyoming, Telephone mines, 1900, Aven Nelson, no. 7833; Colorado, summit of mountains, west of North park, 1899, *G. E. Osterhout.*

**Tradescantia Laramiensis,** n. sp.—Stems 3–4$^{dm}$ long, branching freely; stems and branches erect, stout, glabrous; the branches arising from the axils of the leaves: persistent radical leaves few, linear, 5–10$^{mm}$ wide; cauline leaves linear, forming a short turgid sheath at the base, a little narrower than the radical leaves; both radical and cauline strongly divaricate (nearly at right angles to the stem); the involucral leaves narrow (2–3$^{mm}$ wide), often equaling the cauline: umbels sessile, terminating stems and branches: flowers very numerous in each umbel (often 50), hanging down in age: pedicels long (2.5–3.5$^{cm}$): pedicels and sepals remarkably glandular-pubescent: sepals linear-oblong, acute, 10–12$^{mm}$ long: petals dark blue, broad, obtuse, about double the length of the sepals: filament once or twice sharply folded on itself and immersed in the copious wool arising from its lowest segment: ovary ribbed with rows of glandular hairs.

*T. Laramiensis* is related to *T. scopulorum,* but differs from it in the color of the flowers (*T. Laramiensis* being much darker), in stoutness, in the size and the arrangement of the umbels, in glandulosity of the inflorescence and flower, and in fruit characters. The remarkable pubescence is alone enough to separate *T. Laramiensis* from *T. scopulorum,* but the woolly condition of the filaments and enormous umbels are also decisive characters.

Collected in moist, rocky ground in Halleck cañon, Albany co., Wyoming, by *Aven Nelson,* no. 7455.

**Iris pelogonus,** n. sp.—Roots thick and fleshy: rootstock stout: stem simple, terete, stout, 2.5–3.5$^{cm}$ long, 1–2-flowered: leaves mostly basal, very dull light green, thick and rigid, half as long to a little longer than the scape, 3–6$^{mm}$ wide: flowers in bud dark blue, fading in age, pediceled; pedicel 1–10$^{mm}$ long: perianth segments prominently nerved, glabrous, not crested, simple, outer ones 4–6$^{cm}$ long, about 2$^{cm}$ wide; the inner a little shorter and about 8$^{mm}$ wide: tube above ovary 8–12$^{mm}$ long: stigmas 12–18$^{mm}$ long, exceeding the filaments by 5 or 6$^{mm}$: bracts scarious, lanceolate-acuminate, always completely concealing the ovary and usually extending half the length of the perianth segments.

This is a peculiar plant found in the Wasatch Tertiary clays. Its nearest relative is *I. Missouriensis* from which it differs in size, being much smaller, in the marked difference in color, width, and rigidity of the leaves, stoutness of the whole plant, being much stouter in proportion to its size, in the com-

parative length of the bracts with the pedicel, ovary, and flower, and in the relative length of the stigmas and filaments.

Collected at the Bush ranch, Sweetwater co., Wyoming, by *Aven Nelson*, no. 7102.

**Alsine validus,** n. sp.—Plant glabrous, much branched: stems four-angled, 12–18$^{cm}$ high: leaves lanceolate, broadest at base, 1–3$^{cm}$ long, thick, subcoriaceous, acute, rather rigid, never ciliated at base: bracts very small, 1–3$^{mm}$ long, ovoid or oblong, somewhat acute, scarious: flowers in a usually terminal, many-flowered, compound cyme; pedicels and rays subequal, very variable, 2–5$^{cm}$ long; pedicels straight and rigid, spreading or horizontal: petals deeply two cleft, a little exceeding the sepals: sepals ovoid, with somewhat acute point and scarious margins, 2–3$^{mm}$ long, 1.5–2$^{mm}$ wide: capsule dark brown, shining, nearly twice the length of the sepals, oval, obtuse: styles 3, 3–4$^{mm}$ long, recurved and crested with an abundance of very fine short bristles: seed quite smooth.

The many-flowered compound cyme, stout, thick, wide-spreading pedicels, obtuse capsule, and very small bracts readily separate *A. validus* from *A. longipes*, its nearest relative. The latter has a simple few-flowered cyme, filiform, erect pedicels, more or less acute and more elongated capsule, and also much larger bracts. The character of the inflorescence of *A. validus* is much like that of *A. longifolia*, except that the pedicels are straight and stout instead of filiform and curved.

It was collected in the Centennial valley, Albany co., Wyoming, by *Aven Nelson*, and is the only material of it at hand. The type no. is 7726.—LESLIE N. GOODDING, *University of Wyoming, Laramie.*

# CURRENT LITERATURE.

## BOOK REVIEWS.

### Plants in their haunts.

ALMOST simultaneously three attractive books have appeared, having for their mission the acquaintance of laymen and amateurs with plants in their natural field relations.[1] *Flowers and ferns in their haunts* is a particularly attractive volume, the full page engravings being unusually clear and true to nature, while at the same time they are artistic gems. The chapters introduce the reader to various topics, from the coming of spring to the aftermath, and many of the topics bring out various ecological features in a purely non-technical manner. Some of the more suggestive chapter headings in this regard are "Along the waterways," "Poisonous plants," "In silent woods," "Flowers of the sun," "Wayfarers."

*Our ferns in their haunts*, by the well-known student of ferns, Willard N. Clute, aims to present ferns to beginners and fern-lovers in such a way that they will learn to know them easily and yet in a scientific manner. The book is profusely illustrated and contains very much more detailed accounts of both structure and habitat than are to be found in the ordinary manuals. Much in the way of folk lore is added as well. The keys and glossary seem so well gotten together that the identification of ferns must surely be made easier than that of seed plants.

*With the wild flowers* is a neat little volume that has attained a second edition. It does not stick too closely to scientific facts at all points, as in the cut showing leaf structure. One could wish also that such small and relatively harmless plants as the sun-dew were not included under the blood-curdling title of "ogre-flowers." The impersonation of plants is carried to an extreme and even hurtful degree in this volume.—H. C. COWLES.

[1] WRIGHT, MABEL OSGOOD: Flowers and ferns in their haunts. 8vo. pp. xix + 358, with 57 full page photographs and 118 text illustrations drawn from photographs. New York: The Macmillan Company. 1901.

CLUTE, WILLARD N.: Our ferns in their haunts. 8vo. pp. xii + 332, with 8 colored plates and 185 text cuts and full page plates. New York: Frederick A. Stokes Company. 1901.

GOING, MAUD: With the wild flowers. 16mo. Revised edition. pp. xiv + 271, with 55 text cuts and full page plates. New York: The Baker and Taylor Company, 1901.

## MINOR NOTICES.

IN DRYER'S new "Lessons in physical geography"[2] we are pleased to notice a fuller account than is usual in such books under the topic "Plant geography." It is refreshing to see some of the more cogent ecological facts presented in place of the time-worn statements current in the older physical geographies.— H. C. COWLES.

THE FIFTH AND SIXTH parts of Engler's *Pflanzenreich*[3] have appeared, continuing the finely organized and illustrated presentation of the previous parts. Rafflesiaceae (fam. 75) comprise 7 genera and 27 species, and Hydnoraceae (fam. 76) 2 genera and 10 species. Symplocaceae (fam. 242) are represented by the single great genus Symplocos, containing 282 species, 114 of which are described as new.— J. M. C.

THE FIRST FASCICLE of the fifth volume of Thomé's *Flora von Deutschland*,[4] dealing with cryptogams, has appeared, with Dr. Walter Migula as author. The pteridophytes appeared in the first volume, so that the present one begins with the Bryophytes. A general discussion (21 pp.) of the essential features of the group, illustrated by the excellent plates, precedes the systematic presentation. The fascicle includes the Sphagnaceae, comprising 22 species of Sphagnum, and also the beginning of the Andreaeaceae.— J. M. C.

DR. EUG. WARMING[5] has published his sixth contribution dealing with Podostemaceae, presenting the genera Polypleurum, Cladopus, Griffithella, Sphaerothylax, Tristicha, and Marathrum. The very full and illustrated account of the anatomy, morphology, and distribution of these interesting forms follows the method of the preceding papers, which appeared in the same publication in 1881, 1882, 1888, 1891, and 1899. In another contribution the author promises to present a systematic revision of the family, based upon these studies.— J. M. C.

DR. K. GIESENHAGEN[6] has published a very complete monograph of *Niphobolus*, a genus of epiphytic ferns of the oriental tropics and subtropics.

[2] DRYER, CHARLES R.: Lessons in physical geography. 12mo. pp. 430, with 347 figures. New York, Cincinnati, Chicago: American Book Company. 1901.

[3] ENGLER, A.: Das Pflanzenreich. Regni vegetabilis conspectus. Part 5. Rafflesiaceae und Hydnoraceae von H. Graf zu Solms-Laubach. *M* 1.40. Part 6. Symplocaceae von A. Brand. *M* 5. Leipzig: Wilhelm Engelmann. 1901.

[4] Flora von Deutschland. V. Kryptogamen-Flora. Lfg. 1. pp. 1–32, with 7 plates, 5 of them colored. Gera: Friedrich von Zezschwitz. 1901. *M* 1.

[5] Familien Podostemaceae. VI. Memoires de l'Academie royale des Sciences du Danemark. Danske Vidensk. Selsk. Skrift. 11: 1–67. 1901 (part 1).

[6] Die Farngattung Niphobolus. 8vo. xii+223, with 20 text figures. Jena: Gustav Fischer. 1901. *M* 5.50.

In the first chapter (pp. 1–11) he discusses the general principles of the classification of ferns; in the second (pp. 12–30) a history of the genus is given; and in the third (pp. 21–85) very full details of its morphology are presented, under the headings prothallium, rootstock, root, leaf forms, trichomes, venation, hydathodes, sori, and leaf anatomy. The fourth chapter (pp. 86–223) contains detailed descriptions, both diagnostic and supplementary, of the 49 species, 10 of which are described as new.—J. M. C.

DR. JAKOB HUBER, botanical director of the Museum of Natural History of Para, has issued the first two decades of his *Arboretum Amazonicum*.[7] Each species considered is represented by at least a page of text in Spanish and French in parallel columns, and a plate. The plates are exceptionally fine, being heliotypes from remarkably good photographs. Many of the plants are represented in their natural setting, and the views of tropical plant formations are the finest we have seen. This series of illustrations will be highly prized for its artistic and scientific excellence. In addition to illustrations of prominent genera, several plates represent characteristic plant societies, as a savanna and two illustrations of river bank vegetation. The work will appear in ten parts, at 10 francs a part. The author is to be congratulated upon this valuable contribution to botanical literature.—J. M. C.

ANOTHER FASCICLE of *Plantae Bakerianae*[8] has just appeared. It contains "a somewhat miscellaneous congeries of paragraphs dealing with new or otherwise interesting species" of Mr. Baker's collecting in the Gunnison watershed in the summer of 1901. It is a first installment of volume III of the series, and is published before the completion of volumes I and II. It contains presentations of Ranunculaceae (n. spp. of Cyrtorhynca, Delphinium, and Aconitum), Cruciferae (n. spp. of Draba, Arabis, and Thelypodium), Violaceae (6 n. spp. of Viola), Polygonaceae (n. spp. of Polygonum, Rumex, and Eriogonum), Apocynaceae (2 n. spp. of Apocynum), Asperifoliae (n. spp. of Mertensia and Oreocarya), Labiatae (n. sp. of Monardella), Scrophulariaceae (n. spp. of Castilleia and Pentstemon), Compositae (n. spp. of Senecio, Arnica, Helianthus, Tetraneuris, Psilostrophe, Hymenopappus, Artemisia, and Erigeron), Plantaginaceae (n. sp. of Plantago), Nyctaginaceae (n. spp. of Abronia and Allionia), and Papilionaceae (n. spp. of Thermopsis and Lupinus).—J. M. C.

B. T. P. BARKER[9] has described a conjugating process that precedes spore-formation in an undescribed yeast organism obtained from commercial ginger. He describes the characters obtained from plate-cultures and streak-

[7] Arboretum Amazonicum. Iconographie des plantes spontanées et cultivées les plus importantes de la région Amazonienne. Decades 1 and 2. 4to. Para. 1900.

[8] GREENE, EDWARD L.: Plantae Bakerianae. **3**: viii+36. 18 N 1901. [Fasc. I.]

[9] A conjugating "yeast." Phil. Trans. Roy. Soc. B. **194**: 467–485. *pl. 46.* 1901.

cultures, but chief interest centers in the spore-formation. Under special nutritive conditions ("starvation") two neighboring cells put out beaks towards one another, which meet and fuse by their tips. The "compound cell" thus formed appears as two ordinary cells attached to one another by an elongated neck. "A few hours after fusion, in each compartment of the compound cell" the spores begin to round off. The author also obtained appearances that he interprets as fusion of the "nuclear apparatus" in the connecting tube. He discusses the various possible interpretations, but concludes that this phenomenon is a sexual process of the simplest kind. He also proposes a new genus to include this form, and suggests the name *Zygosaccharomyces*. What constitutes sexual fusion, as distinct from other fusions, is a question that is becoming increasingly difficult to answer.—J. M. C.

## NOTES FOR STUDENTS.

CZAPEK shows[10] that the well known transformation of starch to sugar, which accompanies a lowering of temperature, especially in autumn, can be prevented if the sugar concentration is sufficient. Starch was also formed in guard cells of certain plants immersed in a 10 per cent. cane sugar solution at a temperature of 0°.—H. C. COWLES.

TUCKER and TOLLENS,[11] also Fruhwirth and Zielstorff,[12] have taken up the question of the autumnal migration of carbohydrates, proteids, phosphoric acid, and potash from the dying leaves to the perennial stems of plants. The works of Wehmer and Behrens have given rise to a disbelief in such migrations, Behrens attributing losses of these substances to decomposition and leaching out by rain water. The present authors, however, guarding against such processes, conclude that there are fall migrations of useful substances, though the amount of translocated material is very much less than was once believed.—H. C. COWLES.

D. A. ANDREWS has been investigating karyokinesis in the pollen mother cells of Magnolia and Liriodendron.[13] His conclusions in reference to the first mitosis are that the chromosomes arise from the resting nucleus as irregular masses, without a previous formation of the usual uniform spiral, that the resulting chromosomes are mostly U-shaped, and that they divide longitudinally. The identity of the chromosomes, therefore, is not maintained from the first to the second mitosis, the chromosomes in the latter case arising by the segmentation of an irregular spirem and being at first lumpy bodies that assume the form of a shallow U.—J. M. C.

[10] Ber. d. deutsch. bot. Gesell. **19**: 120–127. 1901.

[11] Jour. für Landw. **48**: 39–64. 1900. [12] Landw. Versuch. Stat. **55**: 9. 1901.

[13] Karyokinesis in Magnolia and Liriodendron, with special reference to the behavior of the chromosomes. Bot. Centralb. Beih. **11**: 134–142. *pl. 1.* 1901.

HATTORI[14] has added another to the numerous contributions on the toxicity of copper. Seedlings of several conifers growing in pots endured much more copper than branches in aqueous solutions. Moist air, by decreasing the transpiration, enabled plants to endure a greater concentration in the water—according to very meager experiments. The copper in water distilled from copper vessels was found sufficient to kill roots. The growth of Aspergillus and Penicillium was stimulated by dilute copper; in the case of Aspergillus this did not interfere with the formation of conidia. In all cases the concentration of copper is given in the extremely inconvenient form of a per cent. of $CuSO_4 + 5H_2O$.—E. B. COPELAND.

BENECKE[15] has reinvestigated Cakile and Salicornia in order to test the recently expressed view of Diels that these plants decompose considerable quantities of NaCl in their metabolic activities. Diels observed gradual decrements of salt in distilled water cultures, especially in Cakile, and he supposed that sodium malate, or some such substance, was formed, setting free the chlorine. Benecke thinks that Diels failed to account for the increase of other substances, especially water, in his plants, and that consequently his percentage comparisons were wrong. The present author uses similar methods, and concludes that the chlorine content suffers no decrease in distilled water cultures. Apparent decrease may be due to increase of water, as noted above, and also to variations in chlorine content in leaves of different ages, the old leaves being relatively poor in NaCl.—H. C. COWLES.

IN A RECENT PAPER, Professor Guignard[16] has described the details of of double fertilization in *Naias major*, which do not differ essentially from those in other forms recently described. The male nuclei are elongated, but do not take on the vermiform appearance so conspicuous in the Compositae. One synergid disintegrates soon after the entrance of the pollen tube, and the other remains intact for some time after the fertilization of the egg, and may itself be fertiiized instead of the endosperm nucleus. In the latter case the endosperm does not develop. The fertilized egg immediately divides, one figure showing the fertilized endosperm nucleus in the spirem stage, while the embryo is two-celled. In all previously described cases of double fertilization the endosperm nucleus invariably divides before the fertilized egg shows any signs of division. In many instances two embryos were observed side by side, with the unfertilized endosperm nucleus lying between them. Two of the antipodals soon show signs of breaking down, but the upper one

[14] Studien über die Einwirkung des Kupfersulfats auf einige Pflanzen. Jour. Coll. Sc. Imp. Univ. Tokyo **15**: 371–394. 1901.

[15] Jahrb. für wiss. Bot. **36**: 179–196. 1901.

[16] La double fécondation dans le *Naias major*. Jour. de Bot. **15**: 1–9. *figs. 1–14*. 1901.

continues to enlarge for a long time after fertilization. The chromosomes are very long, the gametophyte number being six, the smallest number yet reported in seed-plants.—W. J. G. LAND.

INVESTIGATIONS dealing with the influence of the medium on plant development continue to multiply. Pethybridge[17] has experimented with various salt solutions on the development of wheat, finding that the dilution of nutrient solutions or the addition to them of NaCl causes root elongation, decrease in leaves, shoots, and root diameter, and increase in the thickness of endodermis walls; the stomata on the under leaf surface almost wholly disappear. Root hair formation is repressed in NaCl solutions and in increased light. Arker[18] finds that the rapidity of growth of the roots of *Lupinus albus* is facilitated by introducing air currents, especially if somewhat rarefied, into the medium. Beauverie[19] found that an increase of osmotic pressure caused a reduction in the aerial portions of fungi, together with a lateral dilatation of the cells; in very strong solutions the entire plant often became submerged. A more recent study of various seed plants has yielded similar results. Beauverie refers the well known root curvature in water to differences in osmotic pressure rather than to aerotropism, finding that the roots grow straight down in concentrated solutions. The aerial axis becomes reduced in height and broadened laterally as in fungi. The anatomical structure is affected also; a thick cork layer is developed very early in strong solutions; but this is not the case where the pressure is weak.—H. C. COWLES.

BERNARD[20] has presented to the Paris Academy of Sciences two papers which will be certain to incite interest and further investigation. In the first paper, entitled precocious tuberculization in plants, he recalls the production of tubercles on legume roots and coralloid processes on various tree roots through stimulation by bacteria and fungi. Bernard shows the remarkable resemblance between the tuberculous organs of lycopods and orchids, two widely separated families. The gametophyte and sporophyte of Lycopodium and the sporophyte of several orchids show essentially similar organs, and are infested by similar fungi, and in all cases the fungus is Fusarium or a related form. Of a large number of tuberous plants investigated by Stahl in his recent mycorhiza studies, Corydalis alone is found to be without fungi. In his second paper Bernard makes the surprising statement that it is his belief that the tubers of the potato are essentially galls and due to fungus infection. He shows that *Fusarium Solani* is always present in the tubers, and it seems likely that this fungus causes the arrest of the terminal bud and

[17] Inaugural dissertation. Göttingen. 1899. (See Bot. Centralb. **87**: 235. 1901.)

[18] Inaugural dissertation. Erlangen. 1900. (See Bot. Centralb. **87**: 235. 1901.)

[19] Compt. Rend. **132**: 226–9. 1901.

[20] Compt. Rend. **131**: 626–629. 1900; **132**: 355–357. 1901.

the development of hypertrophied tissues, which become filled with starch. The author's experiments, while not yet conclusive, strongly support his theoretical conclusions, since a decided parallelism is seen to exist between the amount of tuber formation and the development of the fungus. However, no cultures entirely free from fungus have yet been made. Bernard notes than when the potato was introduced into France, tubers could not be produced from seed cultures, presumably because *Fusarium Solani* did not then infest the soil.—H. C. COWLES.

TANSLEY and CHICK[21] have made some interesting anatomical studies on the conducting tissue system of bryophytes. The main purpose of the investigation was to shed light on the probable origin of conducting tissues in plants. In general the authors confirm the work of Haberlandt, who showed that the Polytrichaceae have a more complex conductive system than some so-called vascular plants. The tracheids of the liverwort Pallavicinia were studied and were shown to conduct eosin solutions more rapidly than neighboring tissues, though much more slowly than Haberlandt found to be true in moss bundles. The rhizome of Polytrichum was found to have structures resembling the roots of seed plants, viz., a typical endodermis with suberized walls, a pericycle, and a central cylinder with a triarch arrangement of the hydroids and leptoids. It would seem from this that the rhizome of Polytrichum may well be called a root. The aerial stems were found to agree with Haberlandt's description. The authors think from the liverwort evidence that demands for more efficient conduction were first met by cell elongation and lignification of the walls. This idea is favored also by experimental evidence, as has been often shown. In most mosses the leaf and stem bundles are not connected, indicating a possible double origin of conducting bundles. Mnium and Bryum show incomplete connections, while Polytrichum and its allies show bundles of hydroids and leptoids permeating the entire plant, as in ferns and seed plants. In Polytrichum there is a hydrome mantle outside the central cylinder of leptome and hydrome, in this respect resembling ferns. The authors think that this outer mantle may be the downward projection of the leaf bundle, the original stem bundle being at the center.—H. C. COWLES.

THE STRUCTURAL DIFFERENCES between the white and green parts of variegated leaves has been recently noted.[22] The general subject of the "panachirung" (perhaps this term may be rendered into English as "albescence") of leaves has had considerable investigation of late. Timpe[23] finds that the anatomical features are much as noted in the review of Rodrigue's paper; in some cases (as Abutilon) the albescent portions are thicker than the

[21] Annals of Botany **15**: 1–38. 1901. [22] BOT. GAZ. **31**: 209. 1901.

[23] Inaugural dissertation. Göttingen. 1900. See Bot. Centralb. **85**: 75. 1901.

green portions of the leaf. Thin albescent parts are due to a reduction in palisades and intercellular spaces. The author finds a close relation to exist between chlorophyll development and leaf thickness, including the development of palisade cells; if chlorophyll stops abruptly in a variegated leaf, the thin part begins abruptly and palisades cease at once; if the chlorophyll fades out gradually, the leaf gradually becomes thinner and palisades gradually cease. These results and those of Rodrigue seem to throw doubt on Stahl's theory that palisades are due to the direct influence of light, and relate them rather to the presence of active synthesis; the reviewer, however, has observed palisade cells in the albescent parts of many leaves. Timpe finds that albescent portions of leaves redden more than the green portions; this harmonizes well with Overton's conclusions,[24] since the white leaf parts are found to be rich in tannins and sugars, though poor in starch.

Laurent,[25] discusses the origin of albescence in plants. There are two types, those coming from spores, and those that reproduce by seed. The latter type is ordinarily thought to be not responsive to external factors. In some situations plants which are commonly albescent have green leaves, and in other situations the reverse is the case. Laurent thinks that some enzyme intervenes to cause a modification in the distribution of the chlorophyll.

Molisch[26] observed that *Brassica oleracea acephala* becomes albescent in cold greenhouses in winter, and becomes green again in summer, the whitening beginning in October and reaching its maximum in February. The author thus holds with Laurent that albescence may be affected by external factors. In the case of Brassica the author thinks that temperature is the factor involved, since warm greenhouse cultures do not show the phenomenon.— H. C. COWLES.

ITEMS OF TAXONOMIC INTEREST are as follows: F. STEPHANI (Bull. Herb. Boiss. II. **1**: 1141. 1901) has segregated a new genus (*Cuspidatula*) of liverworts from Anastrophyllum, the 4 species included belonging to the East Indian and Australian region.— B. L. ROBINSON (Rhodora **3**: 270–276. 1901) has published a synopsis of the North American species of *Euphrasia*, recognizing 7 species, 2 of which are new (*E. Williamsii* from Mt. Washington and *E. Randii* from Mt. Desert.)— THEO. HOLM (Ottawa Nat. **15**: 175–183. *pls. 11–14.* 1901) has described 3 new Canadian species of *Gentiana* belonging to § Crossopetalae.—S. B. PARISH (Proc. Calif. Acad. Sci. III. Bot. **2**: 159–172. 1901) has revised certain troublesome species of *Solanum* of the *S. Xanti* and *S. umbelliferum* group, describing 3 new species and 4 new varieties, and giving detailed lists of collections.— HELGI JÖNSSON (Botanisk Tidsskrift **24**: 127–155. 1901) has published an account of the Rhodophyceae as the first of a series of papers on the marine algae of Iceland, 2 new species

[24] BOT. GAZ. **27**: 491. 1899. [25] Bull. Soc. Roy. Bot. Belg. **39**: 6–9. 1900.

[26] Ber. deutsch. bot. Gesell. **19**: 32–34. 1901.

being described.—JOHS. SCHMIDT (*idem* 157–221. *pls. 2–4*) has published Part IV of his *Flora of Koh Chang* (Gulf of Siam), W. and G. S. WEST contributing the fresh water Chlorophyceae (121 spp., 9 new), TH. REINBOLD the marine algae (62 spp., 2 new), M. GOMONT the Myxophyceae hormogoneae (27 spp., 2 new), and JOHS. SCHMIDT the Peridiniales (44 spp.), *Ostreopsis* being a new genus.—F. V. COVILLE (Proc. Wash. Acad. Sci. **3**: 569–576. 1901) has segregated two new genera from Cassiope, *Harrimanella*, containing *C. stelleriana* DC. and *C. hypnoides* D. Don, and *Arcterica*, containing *C. oxycoccoides* Gray.—P. A. RYDBERG (Bull. Torr. Bot. Club **28**: 605–643. 1901) has published a monograph of the American species of *Limnorchis* and *Piperia*, both genera having been separated by him from Habenaria, in the former genus 24 species being recognized (5 new), and in the latter 9 (3 new).—G. E. OSTERHOUT (*idem* 644–645) has published new species of Linum, Mentzelia, Artemisia, and Agoseris from Colorado.—A. ENGLER (Bot. Jahrb. **30**: 289–445. *pls 9–22*. 1901), in continuing his studies of the African collection of W. Goetze, has published, in addition to numerous new species, a new genus (*Stenadenium* Pax) of Euphorbiaceae.—RUDOLF WAGNER (Œsterr. Bot. Zeitsch **51**: 465. 1901) has described a new genus (*Cyphochlaena*) of grasses from Madagascar, of the tribe Arundinelleae.—J. M. C.

THOMAS[27] has made a comparative and experimental study of subterranean leaves or scales. They correspond morphologically to sheaths, petioles, or leaf blades, and in all cases differ widely from their morphological homologues. This divergence is particularly great where scales correspond to leaf blades, the palisades, lacunae, bundles, mechanical cells, and epidermal cuticle being much reduced or absent. When aerial branches are made to grow in the soil, leaf primordia develop into structures whose anatomy is like that of scales; leaf blades are reduced, petioles are less reduced, or sometimes even greatly enlarged as in Trifolium, channeled petioles change to flat organs, collenchyma and bundles are reduced, the parenchyma is more compact and with polygonal, instead of rounded cells. The upper epidermal walls are less cutinized, and the lower walls more cutinized, than in normal leaves, stomata are entirely lost, palisades and air spaces are reduced, and reserve foods are stored in abundance. When subterranean branches are made to grow in the air, scale primordia develop into leaves, stomata appear, palisades, vascular tissues, and air spaces are more fully developed.

Some very interesting conclusions are drawn by the author. The scale is found to be the morphological equivalent of the leaf part nearest the base, *i. e.*, the blade in a sessile leaf, the petiole or sheath in a petioled leaf. The changes observed are quite different from the effects of mere darkness, nor are they to be attributed to arrested development, since they differ widely

[27] Rev. Gen. Bot. **12**: 394–404, 417–433. 1900.

from early leaf conditions. The reductions in palisades, air spaces, bundles, and collenchyma are analogous to conditions in young leaves, or to leaves grown in the dark (though the reduction is more complete in the soil); but the entire loss of stomata, the great development of reserve foods, and the strong cutinization of the lower epidermis are without a parallel in other conditions than those furnished by a sojourn in the soil. In some cases, notoriously in *Lysimachia vulgaris*, palisades appear in all conditions, even in leaf primordia while still in the bud and four or five centimeters below ground. The author consequently inclines to agree with Pick that palisades are due to hereditary influences, and that their direction only is determined by light. Thomas also thinks that the changes produced in soil conditions are in direct response to the new needs which arise there.—H. C. COWLES.

STRASBURGER,[28] in a very comprehensive paper, has taken up the whole subject of protoplasmic continuity in plant cells. He proposes the term *Plasmodesmen* for the connecting fibers. Among others, new observations are figured and described for Viscum, Pinus, Phytelephas, Nerium, the sieve tubes of Wistaria and Vitis, leaf cells of mosses, and the cells of grafts of Abies and Picea. But the paper is devoted fully as much to a critical résumé of the literature as to the recording of new observations. To recapitulate all the points made is plainly impossible in a brief review, and only a few of the most important will be mentioned. Kienitz Gerloff's view that the *Plasmodesmen* do not originate in the fibers of the central spindle is confirmed, and they must hence arise secondarily after cell division, but the question as to just how and when they are formed is left unsettled. Figures are given from Pinus and Wistaria confirming the prevalent belief that the thicker connecting strands of the sieve tubes originate as *Plasmodesmen*. As to the functions of *Plasmodesmen*, Strasburger confirms Gardiner's view, that in the endosperm of Tamus they serve to transport enzymes. In the medullary rays of Abies they may serve to transport proteids. That protoplasmic streams in general, however, pass through the fibers, or that they serve, for example, for the withdrawal of protoplasm from the leaves in the fall, is shown to be entirely unproved. The importance of the *Plasmodesmen* for transmitting stimuli and for the normal development of organs is shown by a number of very interesting observations and experiments. Complete plasmolysis results in the withdrawal of the *Plasmodesmen* from their pores in Mnium. Though when washed out the plasmolyzed cells again press upon the cell walls, the *Plasmodesmen* are not reestablished, and the tissue, although it may live several weeks, develops no further and ultimately dies. Plasmolyzed root tips of *Vicia Faba* cannot afterward react geotropically. This may be due to the loss of the *Plasmodesmen*, or to injury of the

[28] Ueber Plasmaverbindungen pflanzlicher Zellen. Jahrb. wiss. Bot. **36**: 493–610. *pls. 14–15*. 1901.

young cell walls. The existence of *Plasmodesmen* between the symbiont cells in grafts of Abies and Picea is shown conclusively. Such facts as that a grafted shoot does not produce roots at its base, and that a grafted lateral twig may bend up and replace a lost central shoot, are doubtless due to morphaesthesia, the stimuli being transmitted through the *Plasmodesmen* of the graft. The question as to whether the *Plasmodesmen* involve continuity or only contact of fibers sent out from adjacent cells is fully discussed, but owing to technical difficulties the facts in the case remain undiscovered.— * * *.

HUGO MIEHE[29] has recently investigated certain cases of nuclear migration in the epidermal cells of some monocotyledons. The fact of the polarity of the epidermal cells that form stoma mother cells had been established by the researches of Strasburger,[30] and more recently by those of Miehe[31] himself. Miehe now finds that by subjecting leaves of *Allium Cepa* or *Hyacinthus orientalis* to a great centrifugal force (2500 × gravity), with the basal ends of the leaves directed outward on the centrifugal machine, the polarity of the cells is exactly reversed, *i. e.*, the stoma mother cells are formed in the basal rather than in the distal part of the epidermal cells. By making stationary the tips of some leaves, to the bases of which small parts of the bulbs were left adhering, and allowing them to grow for some time under favorable conditions, the consequent change in the direction of cell growth produces a corresponding change in their polarity. Miehe thinks that the direction of cell growth is the important factor in determining the position of the stoma cell under ordinary conditions.

Perhaps the most interesting part of the paper, however, is the account of the migration of the nuclei from cell to cell, a process that Miehe found to occur as a response to the stimulus produced by wounds. In such cases the nuclei migrate toward the wounded cells, and when they come near the cell walls put out fine processes that protrude through minute pores in the wall. The whole nucleus passes through such a pore and forms a dense mass on the opposite side of the wall. From the fact that in material killed in Flemming's fluid and stained with the triple stain the denser part of the nucleus (including the pointed processes and the part that has already passed through the wall) stains red, while the less dense part stains blue, Miehe supports Fischer's contention that differentiation in staining is due largely to differences in physical rather than chemical structure of the various

[29] Ueber Wanderungen des pflanzlichen Zellkernes. Flora **88**: 105–142. *pl. 11.* 1901.

[30] Ein Beitrag zur Entwickelungsgeschichte der Spaltöffnungen. Jahrb. wiss. Bot. **6**: 301. 1866.

[31] Histologische u. experimentelle Untersuchungen über die Anlage der Spaltöffnungen einige Monokotylen. Bot. Centralb. **78**: 321, 353, 385. 1899.

bodies. Both the enucleated and multinucleated cells formed by the migration of the nuclei generally die. The neighboring cells then grow into the region thus left vacant. The nuclei generally wander to that part of the cell wall where it is growing most rapidly.

The paper constitutes a very suggestive contribution to a comparatively new field in physiological research. All the material experimented upon was afterwards killed in Flemming's or Carnoy's killing fluid and stained with the triple stain. Such a combination of experimental and histological technique is to be commended as a method by which in many cases more accurate results in the study of the physiology of the cell may be acquired than by the use of either method alone.— H. G. TIMBERLAKE.

TEODORESCO[32] has published some valuable organographic results that should have been noted sooner. The topic of the first paper is the influence of different luminous radiations on form and structure, and the author makes general conclusions of a very satisfactory nature. He finds that in all cases blue light acts most like white light, and green most like the dark, while red light is intermediate. For example, leaves show a maximum of surface in blue light, and a minimum in green, while stems elongate most in green light and least in blue. Some leaves (as in the Crassulaceae) and most petioles agree with stems. Petioles that elongate most in blue light elongate more in white light than in the dark. Palisade cells, chloroplasts, conductive cells, and bark develop best in blue light, least in green. Some roots elongate more in white light than in the dark, of others the reverse is true, while still others are neutral. Blue light, as compared with green light also shows roots to be of three classes. The author holds that blue light increases synthetic energy as compared with red or green light.

The second paper[33] treats of the indirect action of light on stem and leaf. The author grew plants wholly in the light, wholly in the dark, and partly in the dark, hoping to settle as between the view of Sachs that leaves can develop fully in the dark if well nourished by means of other leaves in the light, and the view of Frank and others that light has no such indirect influence. Most experiments confirmed Sach's well-known investigations, leaves growing to a greater size in the dark if other leaves on the same plant were in the light; the leaves were also thicker, and the bundles, mechanical tissues, palisades, and epidermis more developed than on plants wholly in the dark. The stems were longer in partly lighted plants than in either of the other cases, thus agreeing with Sachs. In several lianas, however, Teodoresco finds with Frank that indirect light differs in no respect from total darkness.

In a third paper[34] Teodoresco gives the results of his studies on the

[32] Ann. Sci. Nat. Bot. VIII. **10**: 141–263. 1899.

[33] Rev. Gén. Bot. **11**: 369–397, 430–435. 1899.

[34] Rev. Gén. Bot. **11**: 445–470. 1899.

influence of carbon dioxid on form and structure. Plants were grown in atmospheres almost without carbon dioxid, also in atmospheres containing about 2 per cent. of this gas. Marchantia cultures with but little carbon dioxid showed great reduction in the width and length of thallus, as compared with normal plants; no gemmae were formed, neither the characteristic algoid chlorophyll tissue. Air spaces and chloroplasts were much less abundant, but the colorless parenchyma developed normally. The results are almost exactly those of weakened light. In seed plants Teodoresco finds that so long as seedlings are using reserve foods carbon dioxid retards stem elongation. In older plants carbon dioxid favors the elongation of stems, the enlargement of leaf surfaces, and the rich development of bast, wood, palisades, and air spaces.— H. C. COWLES.

SEVERAL PAPERS have appeared which add considerably to our knowledge of the physiological ecology of chlorophyll and carbohydrate synthesis. Among these the most important are by Griffon. The first paper[35] deals chiefly with the relations between color and the synthesis of carbohydrates. Griffon shows that, although a general ratio exists between the amount and color of the chlorophyll and the amount of $CO_2$ which is broken up, in many cases the chlorophyll present is no measure of the chlorophyll function; for example, in alpine plants the synthesis is greater and in halophytes it is less than the amount of chorophyll would lead one to expect. This result confirms the previous work of Bonnier, and is contrary to the view of Pfeffer. In some green half-parasites and symbiotic saprophytes the gas exchanges associated with respiration are actually greater than those associated with carbohydrate synthesis. The significance of these results is not clear, but the author holds that differences in the cell structure of the chlorenchyma explain some cases. Perhaps the most rational theory, however, is that there are several varieties of chlorophyll, some possessing active synthetic powers and others not; this view agrees with recent spectroscopic studies by Etard, who claims to have found a large number of varieties of chlorophyll, and sometimes two or three in one plant species.

Another interesting set of results has to do with the influence of colors other than green on the synthetic processes. Stahl, Pick, and others have held that the red anthocyan colors favor synthesis, whereas Jumelle and Jönsson have held that they retard it. Griffon thinks that they have no influence whatever. Although red leaves commonly do less chlorophyll work than green leaves in related species, this is due to the generally smaller amounts of chlorophyll in red leaves. Red leaves rich in chlorophyll are as active as green leaves of the same or similar species. Nor does the author regard anthocyan as prejudicial in chlorophyll formation, even though red leaves are so

[35] Ann. Sci. Nat. Bot. VIII. 10: 1–123. 1899.

frequently poor in chlorophyll. Griffon agrees with Overton [36] that the autumnal colors are due to excessive carbohydrate concentration in the chlorenchyma. He also holds with Bonnier that the synthetic power of xanthophyll is relatively inconsequential. The influence of various external factors on carbohydrate synthesis is well presented but must be omitted here, except to state that Griffon finds that chlorophyll formed in the dark (as in conifer seedlings) acts normally. In this connection it may be noted that Bouilhac [37] has found chlorophyll in Nostoc by spectroscopic tests and has succeeded in observing the formation of chlorophyll in this form in the dark, when it is fed with carbohydrates, such as glucose. Griffon thinks that the development of chlorophyll in the dark is in all cases associated with a supply of reserve foods.

In his second paper [38] Griffon measured the amount of carbohydrate synthesis in solar light which has traversed one or more leaves. That certain light rays are cut off in traversing leaves is well known, but few previous attempts have been made to measure synthesis. Nagamatz used the starch method, which is now known not to be accurate. Griffon uses the gas method, and finds that active synthesis takes place in the second leaf even where light has traversed a thick and densely green leaf like Hedera or Laurus; in the case of such plants, where a leaf is shaded by two leaves, respiration commonly exceeds synthesis. The synthethic energy of the second leaf varies, according to differences in amount of chlorophyll and chlorenchyma, and to undetermined factors (probably specific chlorophyll differences), between one-half and one-forty-eighth of that in the leaf which receives unmodified solar rays. In diffuse light the synthethic energy of the second leaf is of course still less. To summarize, respiration generally exceeds synthesis where light has traversed two leaves in the sunlight or one leaf in the shade.

Linsbauer [39] has investigated the transparency of a number of leaves with the use of Wiesner's photometric methods. Only the more refrangible rays were studied. Leaves vary widely in the amount of light that they transmit, sun leaves of Cytisus and Cornus transmitting only 0.0003 of the light offered them, while shade leaves of Fagus transmit 0.02. In general, shade leaves transmit much more light than do sun leaves. The same species and even the same leaf (as N. J. C. Müller showed) shows wide variations, the shade leaf of Cornus transmitting seven times as much light as the sun leaf; perhaps these differences are due in part to variations in the amount of the

[36] Rev. Gen. Bot. **12**: 209–223, 272–288. 1900.

[37] Bot. Gaz. **27**: 491. 1899.

[38] Comp. Rend. **126**: 1583–1586. 1898.

[39] Bot. Centralb. Beihefte **10**: 53–89. 1901.

products of synthesis which are present. The white portions of variegated leaves absorb much more light than does the chlorophyll alone.

Reference has been made previously[40] to the work of Goldflus who found an amount of synthethic energy in the chlorophyll layer underneath the cork of trees that is surprising in the light of the experiments of Griffon and Linsbauer, as noted above.— H. C. COWLES.

ONION smut[41] and grape rots[42] are the subjects of two bulletins from the Ohio Experiment Station. The smut of the onion is able to penetrate the young plant only while leaving the seed or very soon thereafter. As it has no effect upon the sets, it is perfectly safe to put the latter into an infected field, if they have been grown in a seed bed free from smut spores. In certain parts of Ohio where growing sets from seeds is practiced, the loss from smut has amounted to 25 to 40 per cent., and even more, *i. e.*, to 100 to 200 or more bushels of sets per acre. Preliminary experiments go to show that by sprinkling the seeds, after they are scattered in the rows, with a solution of 1 oz. of 40 per cent. formalin in 2 to 3 gallons of water, until well moistened, and then covering with earth, the loss from smut is very greatly diminished. Ground burnt lime applied in the usual manner at a rate of 150 bushels per acre is also recommended as worthy of trial. Further experiments are to be undertaken to determine the commercial value of these treatments.

The grape rots causing the most damage in Ohio are white rot (*Coniothyrium diplodiella*) and black rot (*Laestedia bidwellii*). The former seems not to have been well distinguished from the latter, which may account for the paucity of the literature on the subject. It is less amenable to treatment, possibly from the fact that it is at its maximum almost immediately preceding ripening of the grapes, at a time when Bordeaux mixture and the more permanent fungicides cannot be used for fear of decreasing the market value of the fruit. It is only by constant spraying, beginning before the flower buds open, and continuing as close to the maturity of the grapes as possible, at least eight or nine sprayings a season, that the best results can be obtained in the grape regions of Ohio.— ERNST A. BESSEY.

[40] BOT. GAZ. 31: 440. 1901.

[41] SELBY, A. D.: Onion smut. Preliminary experiments. Bull. Ohio Agr. Expt. Sta. 122: 71–84. *figs. 1–4.* December 1900. Wooster.

[42] ——— and HICKS, J. F.: Grape rots in Ohio, Experiments in the prevention of grape rot. *ibid.* 123: pp. 85–102. *figs. 1–3.* January 1901.

# NEWS.

DR. EUGEN WARMING has been appointed director of the Geological Survey of Denmark.

DR. BRADLEY M. DAVIS, of the University of Chicago, has returned to his work from a stay in Paris.

AN EXCELLENT PORTRAIT of the late Thomas Meehan was published in *Meehan's Monthly* for December last.

DR. E. B. COPELAND, formerly of the University of West Virginia, is engaged in research work at the University of Chicago.

THE DISTINGUISHED MYCOLOGIST, Professor Robert Hartig, director of the Botanical Institute of Munich, died October 9, 1901.

WE LEARN through *Science* of the recent death of Professor Miguel Colmeiro, director of the Botanical Garden at Madrid, aged 86.

MISS JOSEPHINE E. TILDEN, of the University of Minnesota, has just returned from a brief winter exploring trip on the Vancouver coast.

M. DURAND has been appointed director of the Botanical Garden in Brussels, in place of M. Crépin, who has resigned because of ill health.

DR. CHARLES J. CHAMBERLAIN, of the University of Chicago, is spending the current academic year in Europe, chiefly at the University of Bonn.

PROFESSOR WETTSTEIN of Vienna has returned from his Brazilian trip and reports that he has secured valuable collections from hitherto unexplored regions.

DR. JOHN G. COULTER, formerly of Syracuse University, has been appointed professor of botany in the Manila Normal School, Philippine islands.

DR. RICHARD SADEBECK, professor of botany, and director of the botanical museum and laboratory connected with the Colonial Institute at Hamburg, has recently retired.

THE DIRECTOR of the Botanical Survey of India has projected a series of regional floras; the first of these, dealing with the Bombay presidency, has just been issued.—*Nature.*

THE SOCIETY for Plant Morphology and Physiology held its fifth annual meeting at Columbia University, December 31 and January 1, under the presidency of Dr. Erwin F. Smith.

BENJAMIN H. SMITH has been elected director, John W. Harshberger recorder, and Stewardson Brown curator of the botanical section of the Academy of Natural Sciences of Philadelphia for the ensuing year.

PROFESSOR F. LAMSON-SCRIBNER, chief of the Division of Agrostology of the Department of Agriculture, has been given charge of the Bureau of Agriculture to be organized in the Philippines. He will sail with his family February 1.—*Science.*

MR. K. FUJII, assistant in botany in the Imperial University of Tōkyō, has been sent by his government to Germany for three years' study in morphology and cytology. After stopping for a brief visit to the botanical laboratories of the University of Chicago, he left directly for New York and sailed for Germany.

MR. D. G. FAIRCHILD, who is government expert in plant introduction, is on his way to Ceylon and southern India, expecting to return to China during the summer. He is seeking to discover useful plants that may be grown in America. It is an interesting fact that Mr. Bryan Lathrop, of Chicago, who is responsible for these excursions, occupies the unique position of a man who is spending his money to assist his government in botanical work.

THE NATURALISTS of the central states at their recent meeting in Chicago determined to form a permanent organization, and a committee of five was appointed to suggest a plan of organization, to confer with a similar committee of the American Society of Naturalists regarding the relationship between the two societies, and to report at the next meeting of these organizations, which is to be held at Washington in January, 1903. The committee include two botanists, Dr. J. M. Coulter and Dr. William Trelease.

MR. ELAM BARTHOLOMEW, Stockton, Kansas, has assumed the editorship and publication of Ellis and Everhart's *Fungi Columbiani.* Every effort is promised to make this continued publication of high scientific value. There will be no "a," "b," and "c" packets, but a given species occurring on two or more hosts will in each subsequent issue constitute a new number. It will be considered allowable in some instances, in order to show ecological variation and geographical distribution, to reissue a species on the same host when it comes from widely separated regions. It is expected that two centuries will be issued each year, each issue being limited to seventy copies, and the subscription price being $6.00 a copy unless some previous contract has been made.

THE FOLLOWING persons have been appointed American editors of the reorganized *Botanisches Centralblatt:* D. H. Campbell, morphology; C. J. Chamberlain, cytology; D. T. MacDougal, physiology; G. T. Moore, algae; D. P. Penthallow, paleontology; H. von Schrenk, fungi; William Trelease, phanerogams. In order that the work of the American board of editors may

be properly coordinated Professor Trelease has been asked to act as chairman of the editorial board with the assistance of Dr. von Schrenk as general secretary. The central position of St. Louis and its excellent library and exchanges recommend it as the place most suitable for the editorial headquarters.

The British board of editors is as follows: Miss Edith Barton, algae; George Massee, fungi; Anthony Gepp, archegoniates; B. Daydon Jackson, phanerogams; J. Bretland Farmer, cytology; S. H. Vines, physiology; W. H. Lang, morphology, D. H. Scott, paleontology.

WE NOTE the following list of calls or promotions from the *American Naturalist:* DR. LUJO ADAMOVIC, known for his excellent work in phytogeography, to be professor of botany and director of the Botanical Garden at Belgrade; DR. A. H. R. BULLER to lecture in botany at the University of Birmingham; DR. FREDERIC E. CLEMENTS to an adjunct professorship in botany at the University of Nebraska; MR. GEORGE H. LYMAN to be professor of botany at Dartmouth College, in place of Dr. G. T. Moore, who is now with the Department of Agriculture; W. J. PALLADIN, the well-known physiologist, to be professor of physiology and plant anatomy at the University of St. Petersburg; MR. JOHN J. THORNBER, formerly associated with the Botanical Survey of Nebraska, to be professor of botany at the University of Arizona; DR. OSCAR UHLWORM, who has for so many years been associated with the *Botanisches Centralblatt*, to be librarian of the Royal Library, Berlin, also chief of the German Bureau for International Bibliography.

THE THIRD annual meeting of the botanists of the central states was held at the Hull Botanical Laboratory of the University of Chicago, December 31 to January 2, inclusive. Since there has been as yet no organization effected, there was no complete independent registration of the botanists. More than forty professional botanists, however, were present, and attendance at the sessions varied from fifty to eighty. Even during the last hours, when it was expected that many would have left the city, more than fifty were present. Among the institutions represented were the following: The University of Colorado, Francis Ramaley; the University of South Dakota, M. A. Brannon; the University of Minnesota, Conway MacMillan, H. L. Lyon; the University of Wisconsin, R. A. Harper, H. G. Timberlake, C. E. Allen; Beloit College, H. G. Densmore; State Normal School, Milwaukee, J. N. Mitchell; the University of Iowa, T. H. Macbride; Upper Iowa University, Bruce Fink; Parsons College, F. D. Heald; the University of Missouri, Charles Thom; Missouri Botanical Garden, William Trelease; University of Illinois, T. J. Burrill, C. F. Hottes; Illinois State Normal School, O. W. Caldwell; Illinois College, J. B. Overton; Blackburn University, Charles Robertson; Northwestern College, L. M. Umbach; University of Indiana, D. M. Mottier, C. A. King; Purdue University, Stanley Coulter; University of

Michigan, F. C. Newcombe, J. B. Pollock, H. S. Reed; Michigan Agricultural College, C. F. Wheeler; University of Ohio, E. E. Bogue; Oberlin College, F. O. Grover; Alabama Polytechnic Institute, E. M. Wilcox; Field Columbian Museum, C. F. Millspaugh; Northwestern School of Pharmacy, Albert Schneider; the University of Chicago, J. M. Coulter, C. R. Barnes, B. M. Davis, H. C. Cowles, B. E. Livingston, W. J. G. Land, H. N. Whitford, J. M. Westgate, C. D. Howe, G. H. Shull, F. M. Lyon, T. C. Frye, L. M. Snow, M. E. Mathews, G. E. Yocum, G. M. Holferty; E. B. Copeland, and unattached, E. J. Hill. The program of papers was very full. A list of these and abstracts, so far as furnished by the authors, will be published in *Science*. Thirty-two botanists participated in the annual dinner of the American Society of Naturalists at the Auditorium Hotel, Wednesday, January 1. The third report of the committee appointed by the Society for Plant Morphology and Physiology to secure better reviews of botanical literature was sent by the chairman of the committee to be presented simultaneously to the botanists of the central states. Printed copies were distributed and full verbal explanation of the plans regarding the new management of the *Botanisches Centralblatt* was made by Dr. Trelease. The meeting expressed its appreciation of the courtesy of its eastern confrères in sending copies of the report. On Thursday morning it was decided to form a permanent organization and a committee consisting of Messrs. Coulter, Mottier, and MacMillan was appointed to formulate plans for organization and to present them at the next meeting. It was voted that the next meeting of the central botanists should be held in Washington, D. C., in connection with the meeting of the American Association for the Advancement of Science and the American Society of Naturalists.